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STUDY OF ANTIOXIDANT ACTIVITY OF β-AMINOPROPIOAMIDOXIME O-SULFOARIL DERIVATIVES

Abstract. Amidoxime derivatives have a broad spectrum of biological activity. Herein we report the screening of the products of the interaction of β-aminopropioamidoximes [amino group: piperidin-1-yl; morpholin-1-yl; thiomorpholin-1-yl] with *para*-substituted arylsulfonyl chlorides on antioxidant activity.

Antioxidants of non-enzymatic nature are intended to reduce oxidative stress. The most commonly used antioxidants are vitamin C, vitamin E, quercetin, β-carotene. As a rule, antioxidants «quench» free radicals, easily giving protons to active forms of oxygen: singlet oxygen ($O^{\bullet}$), superoxide anion radical ($O_2^{-\bullet}$), hydrogen peroxide ($H_2O_2^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), peroxyl radical ($R\text{-}COO^{\bullet}$), nitrogen oxide ($NO^{\bullet}$), peroxynitrite ($ONOO^{\bullet}$). We determined the antioxidant activity of O-sulfoaryl derivatives of β-aminopropioamidoximes by the DPPH method using quercetin as a reference. Quercetin is able to stabilize an electron radical by several continued conjugation schemes, including C=C and C= O bonds.

The percentage of inhibition of the DPPH radical by quercetin is 92.3%. Derivatives of β-(morpholin-1-yl)propioamidoxime with methoxy, methyl, and bromo in the *para*-position of the phenyl ring of the sulfogroup lead to 19.31, 11.50, and 10.88% inhibition, respectively; compounds of these series with an unsubstituted phenyl ring and *para*-nitrosubstituent showed inhibition of 7.68% and 5.73% respectively. O-Tosylate β-(thiomorpholin-1-yl)propioamidoxime and O-*para*-chlorophenylsulfo-β-(morpholin-1-yl)propioamidoxime showed minimal inhibition of 2.38% and 3.70% respectively. O-Tosylate β-(piperidin-1-yl)propioamidoxime did not revealed inhibition.

Key words: antioxidants, antioxidant capacity, sulfoderivatives of β-aminopropioamidoximes.

Introduction. A variety of amidoxime derivatives are known to have a broad spectrum of biological activity such as anti-tuberculosis, antimicrobial, antiviral, insecticidal, fungicidal, antidepressant, antidiabetic, etc. [1–5].

Sulfogroup is a pharmacophore, which serves as the basis for sulfonylamide drugs that have been widely used in therapy since the 30s as antibacterial agents (streptocid, norsulfazole, sulfazine, sulfadimezin, etazole, sulfadimethoxine, phthalazole) [6].

Stable sulfonyl amidoximes with antioxidant and lipid peroxidant activity are known [7]. This work presents the results of *in vitro* screening of the products of the interaction of β-aminopropioamidoximes and acid chlorides of substituted sulfoaromatic acids for antioxidant activity.

Antioxidants are substances that can inhibit the process of radical oxidation of organic and high molecular compounds. The topic of free radicals and reactive oxygen-containing particles continues to attract increased attention. Reactive oxygen species [singlet oxygen, superoxide anion radical ($O_2^{-\bullet}$), hydrogen peroxide ($H_2O_2^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), peroxyl radical ($R\text{-}COO^{\bullet}$), nitric oxide ($NO^{\bullet}$), peroxynitrite ($ONOO^{\bullet}$)] induce various free radical oxidative reactions in cells.

Their targets are cell membrane lipids, nucleic acids, proteins, enzymes, DNA molecules. As a result, a wide range of pathogenetic effects can be developed: premature aging, radiation sickness, toxicosis, cardiovascular diseases, various types of malignant tumors, neurodegenerative diseases (Parkinson's, Alzheimer's diseases, etc. [8].

Free radicals occur endogenously during normal metabolic reactions or exogenously, for example, as components of tobacco smoking or from environmental pollution, toxins and radiation. In all viable cells, there are protective mechanisms against the damaging effects of free radicals, reducing the risk of atherosclerosis, cancer, diabetes, arthritis, rheumatism and other diseases. The degree of tissue damage depends on the ratio between the accumulation of free radicals and the number of protective antioxidants.

Antioxidants of an enzymatic nature are synthesized by eukaryotic and prokaryotic cells (superoxide dismutase, catalase and peroxidase) and are the most important (internal) part of the antioxidant system of the body. Due to their action every cell is normally capable to destroy the excess of free radicals [9].

However, when there is an excess of free radicals, the external, non-enzymatic part of the antioxidant system – antioxidants, such as food antioxidants and synthetic such as vitamin C (ascorbic acid), vitamin E [(RRR)-α-tocopherol – the natural and most biologically active form of all tocopherols], quercetin, beta-carotene [10–12] (figure 1):

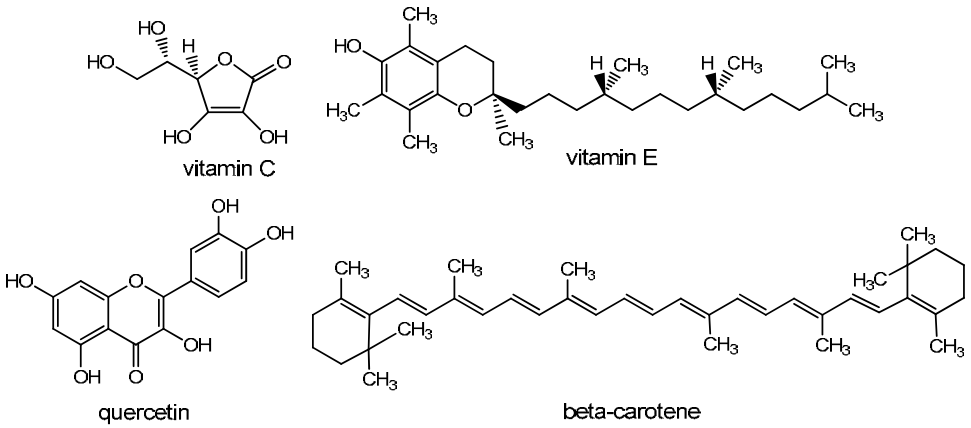
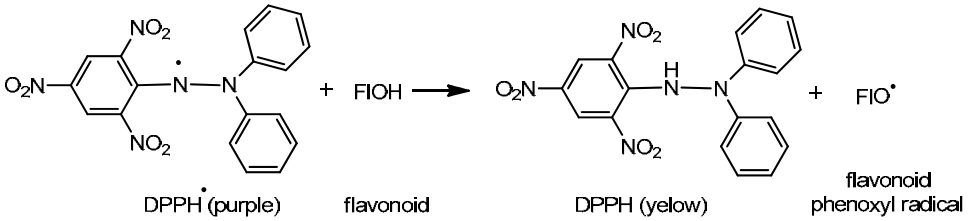


Figure 1 – Non-enzymatic antioxidants

One way to evaluate antioxidant activity is free radical colorimetry, based on the reaction of DPPH (2,2-diphenyl-1-picrylhydrazyl dissolved in ethanol, with a sample of antioxidant according to the scheme: $DPPH^{\bullet} + AH \rightarrow DPPH\text{-}H + A^{\bullet}$ [ ].

In the present work, quercetin was used as a reference antioxidant [13]. The DPPH method is based on the change in the staining of the aqueous-alcoholic media of the test solution when interacting with the antioxidant from violet (λ_{max} ~ 520 nm) inherent in the 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical to yellow, which characterizes the reduced form – 2,2-diphenyl-1-picrylhydrazine (scheme 1).



Scheme 1

Flavonoids, to which quercetin belongs, are capable of stabilizing an electron of the radical with extended resonance stabilization (figure 2).

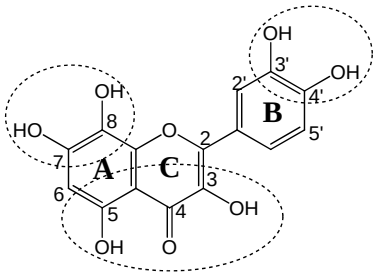


Figure 2 – Structural features of flavonoids with a high radical scavending activity

Essential structural requirements necessary for effective radical stabilization, creating an extended system of multiple bonds – 1) hydroxyl groups in ring **A**, which are donors of proton radicals and increase the ability to absorb radicals, 2) the presence of 3', 4'-dihydroxyl groups in ring **B** flavonoids, 3) C2 – C3 double bond conjugated with the 4-keto group, responsible for the delocalization of electrons in ring **B**; 4) in addition, the 3-OH and 5-OH groups in combination with the 4-carboxyl moiety in ring **C** provide electron delocalization from ring **B** and enhance the ability to absorb radicals.

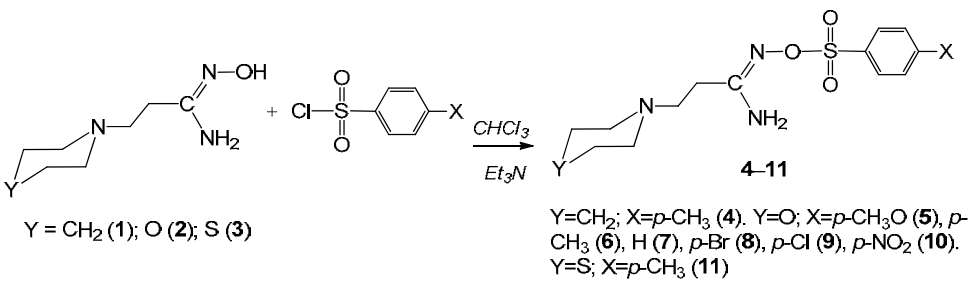
The high potential of flavonoid compounds to capture free radicals ($R^{\bullet}$) can be explained by their ability to release hydrogen atoms from a hydroxyl group and thus «absorb» free radicals:

$$FlOH + R^{\bullet} \rightarrow FlO^{\bullet} + RH$$

This reaction gives a phenoxyl radical ($FlO^{\bullet}$) and a stable molecule (RH). Then $FlO^{\bullet}$ undergoes a change in the resonance structure by redistributing the electron of the radical in the aromatic nucleus. Resonant stabilization of the phenoxyl radical leads to its lower reactivity compared to the radical that received the proton from the flavonoid. $FlO^{\bullet}$ will react further to form non-reactive compounds, probably by radical interaction: $FlO^{\bullet} + R^{\bullet} \rightarrow FlO–R$ and $FlO^{\bullet} + FlO^{\bullet} \rightarrow FlO–OFl$ [14].

RESULTS AND DISCUSSION

We carried out the interaction of β–aminopropioamidoximes [amino group: piperidin-1-yl (**1**); morpholin-1-yl (**2**); thiomorpholin-1-yl (**3**)] with *para*-substituted arylsulfonyl chlorides in chloroform in the presence of an equivalent amount of triethylamine at room temperature for 2–6 days. Based on the elemental analysis, IR and NMR (^{1}H and ^{13}C) spectra, a conclusion about the preparation of a series of O-arylsulfonyl-β-aminopropioamidoximes (**4**–**11**) was made (scheme 2) [15, 16]:



Scheme 2

The reducing properties of the examined compounds **4**–**11** were evaluated by their ability to transform violet 1,1-diphenyl-2-picrylhydrazyl radical (DPP $^{\bullet}$, i.e. DPPH radical) into its pale, yellow reduced form (DPPH). The decrease in absorbance is related to the antioxidant activity of the compound in question (the loss of violet color was measured at 517 nm).

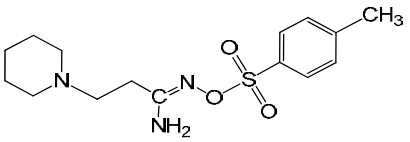
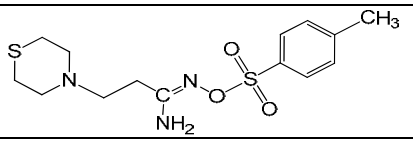
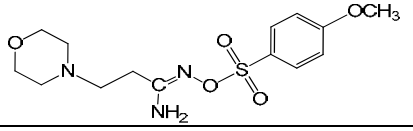
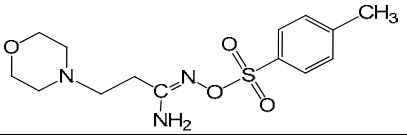
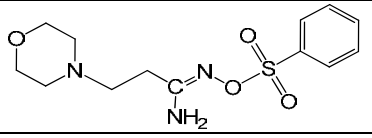
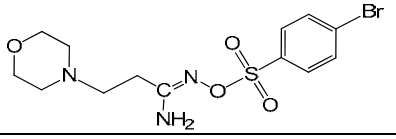
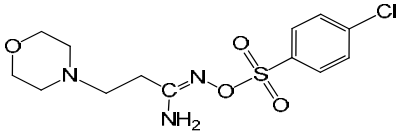
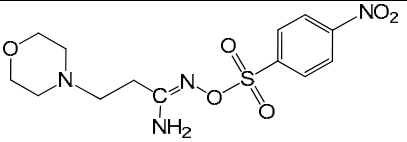
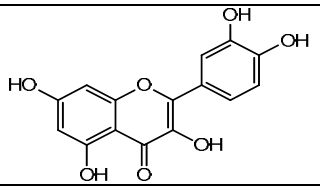
The capacity of the tested substance to reduce DPP• radical to DPPH corresponds to its antioxidant activity and was expressed as a percentage of DPPH radical inhibition [I (%)] according to the following equation:

$$I\% = Ac\text{-}Aa/Ac \cdot 100.$$

Legend: inhibition of DPPH radical – I; absorbance of the control (Ac) and tested samples (Aa).

As a reference compound quercetin with concentration 0.2 mM was used. Quercetin inhibition of DPPH was amounted 92.3%. The results of the examina-

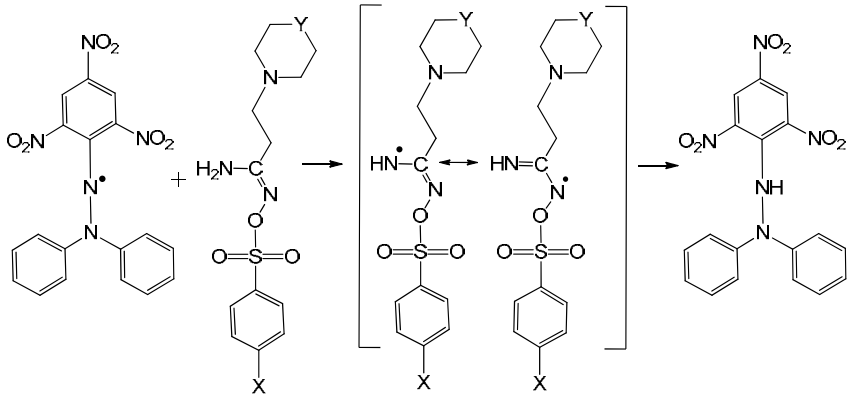
In vitro determined antioxidant activities
of β-aminopropioamidoxime derivatives (**4**–**11**) and quercetin

Comp.	Name	Structure	Antioxidative potential
4	3-(piperidin-1-yl)-N'-(tosyloxy)propanimidamide		0 ± 0.01
5	3-(thiomorpholin-1-yl)-N'-(tosyloxy)propanimidamide		2.38 ± 0.03
6	3-(morpholin-1-yl)-N'--[(4-methoxyphenyl)sulfonyl-oxy] propanimidamide		19,31 ± 0.07
7	3-(morpholin-1-yl)-N'-(tosyloxy)propanimidamide		11.5 ± 0.05
8	3-(morpholin-1-yl)-N'-(phenylsulfonyloxy)pro-panimid-amide		7.68 ± 0.045
9	3-(morpholin-1-yl)-N'-[(4-bromoxyphenyl)-sulfonyloxy]propan-imidamide		10.88 ± 0.05
10	3-(morpholin-1-yl)-N'-[(4-chlorophenyl)sulfonyloxy] propanimidamide		3.70 ± 0.01
11	3-(morpholin-1-yl)-N'-[(4-nitrophenyl)sulfonyloxy] propanimidamide		5.73 ±0.02
Guercetin			92.3± 0.001

tion of antioxidant activity of a number of β-aminopropioamidoximes (**4**–**11**) are given in the table.

According to the equation, the percentage of inhibition of the DPPH radical by quercetin is 92.3%. Compounds **6**, **7**, **9** lead to 19.31%, 11.50% and 10.88% inhibition; compounds **8** and **11** demonstrate inhibition of 7.68% and 5.73%. The minimum inhibition (2.38% and 3.70%) was shown by the compounds **5** and **10**. Compound **4** did not show inhibition.

The most likely mode of action of sulfonic derivatives **4**–**11**, as «radical scavengers», is the path of recoil of a proton from the amino group of the amidoxime fragment and stabilization of the radical in the non-extended chain of atoms: HN•–C=N–O– ↔HN=C–N•–O– (scheme 3).



Y=CH_2; X=*p*-CH_3 (**4**). Y=O; X=*p*-CH_3O (**5**), *p*-CH_3 (**6**), H (**7**), *p*-Br (**8**), *p*-Cl (**9**), *p*-NO_2 (**10**). Y=S; X=*p*-CH_3 (**11**)

Scheme 3

Thus, the experiment showed that a number of sulfonic derivatives **4**–**11** has a lower antioxidant activity compared to the reference compound, quercetin, in which there are significantly more possibilities for the resonant stabilization of the radical in the continuous conjugation chains.

EXPERIMENTAL

IR spectra were obtained on a Thermo Scientific Nicolet 5700 FTIR instrument in KBr pellets. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance III 500 MHz NMR spectrometer (500 and 126 MHz, respectively). The signals of DMSO-d_6 were used as internal reference for ^{1}H NMR (2,50 ppm) and ^{13}C NMR (39,5 ppm) spectra. Melting points were determined in glass capillaries on a PTP(M) apparatus (Khimlabpribor, Russia). The reaction progress and purity of the obtained products were controlled using Sorbfil (Sorbpolymer, Russia) TLC plates coated with CTX-1A silica gel, grain size 5–17 μm, containing UV-254 indicator. The eluent for TLC analysis was mixture benzene–EtOH, 1:3. The reagents were purchased from different chemical suppliers and were purified

before use. The solvents for synthesis, recrystallization, and TLC analysis (ethanol, 2-PrOH, benzene, DMF, acetone, diethyl ether) were purified according to the standard techniques.

Synthesis of the starting compounds are described earlier: β-(piperidin-1-yl)propioamidoxime (**1**), β-(morpholin-1-yl)propioamidoxime (**2**) in the work [17] and β-(thiomorpholin-1-yl)propioamidoxime (**3**) in the work [18].

Evaluation of β-aminopropioamidoxime derivatives antioxidant activity. The general method is as follows: 100 μl of the stock solution was diluted to 2 ml with ethanol (0.1 mg/2 ml) and mixed with 0.5 ml of DPPH radical solution (0.5 mM), resulting in a final concentration of the tested compound of 0.04 mg/ml. The solutions were incubated for 30 min in a dark place at room temperature. The absorbance was measured at 517 nm. The mixture of ethanol and 0.5 mM DPPH radical solution (1:4, v/v) was used as the control.

Stock solutions of the tested β-aminopropioamidoximes (**4**–**11**) were prepared in ethanol (1 mg/mL). Measurements were repeated five times for chosen concentrations of the test compounds for DPPH assay; the processing of experimental data was performed by methods of mathematical statistics. Values are expressed as mean ± STDev.

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Резюме

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β-АМИНОПРОПИОАМИДОКСИМДЕРДІҢ О-СУЛЬФОАРИЛ ТУЫНДЫЛАРЫНЫҢ АНТИОКСИДАНТТЫ БЕЛСЕНДІЛІГІН ЗЕРТТЕУ

β-Аминопропиоамидоксимдердің О-сульфоарил туындыларының антиоксидантты белсенділігін анықтау DPPH әдісі бойынша кверцетинді эталон ретінде қолданумен жүргізілді. DPPH радикалын кверцетинмен ингибирлеу пайызы 92.3 %-ға тең. β-(Морфолин-1-ил)пропиоамидоксим туындыларының метокси-, метильді және бром топтары *пара*- жағдайда фенилсульфотоптары сәйкесінше 19.31, 11.50 және 10.88 % ингибирленуге алып келеді; осы қатардағы орыналмаспаған фениль сақинасымен және орыналмасқан *пара*-нитро қосылыстар 7.68% және 5.73% ингибирленуді көрсетеді. Ингибирленудің 2.38% және 3.70% минимальды көрсеткішін О-тозилат β-(тиоморфолин-1-ил)пропиоамидоксим және О-*пара*-хлорофенилсульфо-β-(морфолин-1-ил)пропиоамидоксим көрсетті. О-Тозилат β-(пиперидин-1-ил)пропиоамидоксим ингибирленуді көрсетпеді.

Түйін сөздер: антиоксиданттар, антиоксиданттық белсенділік, β-аминопропиоамидоксимдердің сульфотуындылары.

Резюме

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ИССЛЕДОВАНИЕ АНТИОКСИДАНТНОЙ АКТИВНОСТИ О-СУЛЬФОАРИЛПРОИЗВОДНЫХ β-АМИНОПРОПИОАМИДОКСИМОВ

Определение антиоксидантной активности О-сульфоарилпроизводных β-аминопропиоамидоксимов выполнено по методу DPPH с использованием в качестве эталона кверцетина. Процент ингибирования DPPH радикала кверцетином составляет 92.3 %. Производные β-(морфолин-1-ил)пропиоамидоксима с метокси-, метильной и бромогруппой в *пара*-положении фенилсульфогруппы приводят соответственно к 19.31, 11.50 и 10.88 % ингибирования; соединения этого ряда с незамещенным фенильным кольцом и *пара*-нитрозаместителем демонстрируют ингибирование в 7.68% и 5.73%. Минимальное ингибирование в 2.38% и 3.70% проявили О-тозилат β-(тиоморфолин-1-ил)пропиоамидоксима и О-*пара*-хлорофенилсульфо-β-(морфолин-1-ил)пропиоамидоксим. О-Тозилат β-(пиперидин-1-ил)пропиоамидоксима не показал ингибирования.

Ключевые слова: антиоксиданты, антиоксидантная активность, сульфопроизводные β-аминопропиоамидоксимов.