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Д.В. Сокольский атындағы  
«Жанармай, катализ және электрохимия институты» АҚ

# Х А Б А Р Л А Р Ы

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РЕСПУБЛИКИ КАЗАХСТАН  
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**SYNTHESIS AND ANTIRADICAL ACTIVITY OF SUBSTITUTED  
CHALCONES AND THEIR DERIVATIVES**

**Abstract.** The antiradical activity of synthesized chalcones, pyrazolines and flavonones was studied in the article by a method based on the interaction of compounds with the stable chromogen radical 2,2-diphenyl-1-picrylhydrazyl (DPPH or DPPH•).

Data on the antiradical activity of the synthesized chalcones, pyrazolines and flavonones were showed. It was found that among a series of new compounds, some derivatives of (E)-1-(2-hydroxyphenyl)-3-(3-ethoxy-4-hydroxyphenyl) prop-2-en-1-one and 4-[5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-3-il] benz-1,3-diol showed a high antiradical activity for DPPH radical. Results of the studying of an antiradical activity are coordinated with the homolytic O-H bond dissociation energies calculated with a quantum-chemical method of density functional.

Quantum chemical calculations were performed using the Gaussian 09w (Revision D.01) program by the density functional (DFT) method. We used a hybrid B3LYP functionality with a basic set of 6-31+G (d,p). The solvent effect was taken into account within the framework of the polarizable continuum (PCM) model.

Values of the chemical shifts, multiplicity and integral intensity of signals in one-dimensional  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were determined. Homo- and heteronuclear interactions confirming structure of the studied compounds were defined with ( $^1\text{H}$ - $^1\text{H}$ ) COSY and ( $^1\text{H}$ - $^{13}\text{C}$ ) HMQC spectra. Chemical shifts are measured relative to the signals of residual protons or carbon atoms DMSO- $d_6$ . Control over the course of the reaction and the purity of the obtained compounds was carried out by thin-layer chromatography on SilufolUV-254 plates in the isopropyl alcohol-benzene-ammonia system, 10:5:2.

**Key words:** chalcones, pyrazolines, flavonones antiradical activity,  $^1\text{H}$  - and  $^{13}\text{C}$ - NMR spectroscopy.

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## **ФУНКЦИОНАЛДЫ АЛМАСТЫРЫЛҒАН АЛКЕНДЕР МЕН ОЛАРДЫҢ ТУЫНДЫЛАРЫНЫҢ СИНТЕЗІ ЖӘНЕ РАДИКАЛДЫ ЕМЕС БЕЛСЕНДІЛІГІ**

**Аннотация.** Мақалада тұрақты хромоген-радикал 2,2-дифенил-1-пикрилгидразилмен (DFPG немесе DPPH•) қосылыстардың өзара әрекеттесуіне негізделген синтезделген алкендердің, пиразолиндердің және флавонондардың радикалды емес белсенділігі зерттелген. Жаңа қосылыстар сериясының ішінде туындылар (E)-1-(2-гидроксифенил)-3-(3-этоксидрокси-4-гидроксифенил) проп-2-ен-1-он және 4-[5-(4-метоксифенил)-4,5-дигидро-1H-пиразол -3-ил] бенз-1,3-диол радикалды DPPH-ге қарсы жоғары радикалды белсенділікті көрсетті.

Антирадикалық белсенділікті зерттеу нәтижелері тығыздық функционалының кванттық химиялық әдісімен есептелген O-H байланыстарының гомолитикалық диссоциациясының энергиясымен үйлеседі. Кванттық химиялық есептеулер Gaussian 09w (Revision D.01) бағдарламасының көмегімен тығыздық функционалы (DFT) әдісімен жүргізілді. B3LYP

гибридті функционалдығын 6-31+G (d,p) негізгі жиынтығымен қолданды. Еріткіштің әсері полярланатын континуум (PCM) моделі аясында ескерілді.

Жұмыста функционалды түрде алмастырылған алкендер мен олардың туындыларын синтездеу туралы мәліметтер келтірілген. ЯМР  $^1\text{H}$ - және  $^{13}\text{C}$ -спектроскопия әдістерімен синтезделген қосылыстардың құрылымын, сондай-ақ екі өлшемді COSY ( $^1\text{H}$ - $^1\text{H}$ ) және HMQC ( $^1\text{H}$ - $^{13}\text{C}$ ) спектрлерінің деректерін зерттеу. Химиялық ығысулардың мәндері, ЯМР  $^1\text{H}$  және  $^{13}\text{C}$  спектрлерінде сигналдарының мультиплеттілігі және интегралды қарқындылығы бір өлшемді анықталады. Спектрлер көмегімен COSY ( $^1\text{H}$ - $^1\text{H}$ ) және HMQC ( $^1\text{H}$ - $^{13}\text{C}$ ) форматтарында зерттелетін қосылыстардың құрылымын растайтын гомо және гетероядролық өзара әрекеттесулер орнатылады.

Химиялық ығысулар қалдық протондардың немесе DMSO-d<sub>6</sub> көміртегі атомдарының сигналдарына қатысты өлшенеді. Реакция барысын және алынған қосылыстардың тазалығын бақылау изопропил спирті-бензол-аммиак жүйесіндегі SilufolUV-254 тақталарында жұқа қабатты хроматография әдісімен жүзеге асырылды, 10:5:2.

**Түйін сөздер:** алмастырылған хош иісті альдегид, алкен, пиразолин, флавонон, антирадикальді белсенділік.

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## **СИНТЕЗ И АНТИРАДИКАЛЬНАЯ АКТИВНОСТЬ ФУНКЦИОНАЛЬНО ЗАМЕЩЕННЫХ ХАЛКОНОВ И ИХ ПРОИЗВОДНЫХ**

**Аннотация.** В статье изучена антирадикальная активность синтезированных халконов, пиразолинов и флавононов методом, основанным на взаимодействии соединений со стабильным хромоген-радикалом 2,2-дифенил-1-пикрилгидразилом (ДФПГ или DPPH•). Установлено, что среди серии новых соединений производные (E)-1-(2-гидроксифенил)-



3-(3-этокси-4-гидроксифенил) проп-2-ен-1-он и 4-[5-(4-метоксифенил)-4,5-дигидро-1H-пиразол-3-ил] бенз-1,3-диол показали высокую антирадикальную активность в отношении DPPH радикала.

Результаты изучения антирадикальной активности согласуются с энергиями гомолитической диссоциации связей О-Н, рассчитанными квантовохимическим методом функционала плотности. Квантовохимические расчеты выполняли с помощью программы Gaussian 09w (Revision D.01) методом функционала плотности (DFT). Использовали гибридный функционал B3LYP с базисным набором 6-31+G(d,p). Влияние растворителя учитывали в рамках модели поляризуемого континуума (PCM).

В работе приведены данные по синтезу функционально замещенных халконов и их производных. Исследовано строения синтезированных соединений методами ЯМР  $^1\text{H}$ - и  $^{13}\text{C}$ -спектроскопии, а также данными двумерных спектров COSY ( $^1\text{H}$ - $^1\text{H}$ ) и HMQC ( $^1\text{H}$ - $^{13}\text{C}$ ). Определены значения химических сдвигов, мультиплетность и интегральная интенсивность сигналов  $^1\text{H}$  и  $^{13}\text{C}$  в одномерных спектрах ЯМР. С помощью спектров в форматах COSY ( $^1\text{H}$ - $^1\text{H}$ ) и HMQC ( $^1\text{H}$ - $^{13}\text{C}$ ) установлены гомо- и гетероядерные взаимодействия, подтверждающие структуру исследуемых соединений. Химические сдвиги измерены относительно сигналов остаточных протонов или атомов углерода ДМСО- $d_6$ . Контроль за ходом реакции и чистотой полученных соединений осуществляли методом тонкослойной хроматографии на пластинках SilufolUV-254 в системе изопропиловый спирт-бензол-аммиак, 10:5:2.

**Ключевые слова:** замещенный ароматический альдегид, халкон, пиразолин, флавонон, антирадикальная активность.

**Introduction.** Recently the literature has a large amount of data on a key role of free radical oxidation connected with active forms of free oxygen radicals, having a high oxidizing ability (Awasthi et al., 2009:7). These radicals, collecting in cell, lead to a toxic state which is called an oxidative stress. A special role in the binding and reducing of the effect of free radicals is belonged to substances – antioxidants. The search of compounds with an antioxidant activity among chalcones and their heterocyclic derivatives is one of the important applied problems of the modern medical chemistry (Achanta et al., 2006:7). It should be noted that compounds with chalcone fragment have the high antitumoral, antibacterial, antifungal, antiviral, antimalarial, hyperglycemic, anti-inflammatory and immunomodulatory activities, and demonstrate the chemoprotective and antioxidant properties. In addition some chalcone derivatives have an ability to strengthen capillaries (Barford et al., 2002:7).

**Research Material and methods.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of compounds



1-8 were recorded on JNM-ECA Jeol 400 spectrometer (frequency 399.78 and 100.53 MHz respectively) with using of DMSO-d<sub>6</sub> solvent. The chemical shifts were measured concerning signals of residual protons or carbon atoms of DMSO-d<sub>6</sub>. The control of the reaction and purity of the received compounds was performed by Thin Layer Chromatography method on Silufol UV-254 plates in isopropyl alcohol-benzene-ammonia system (10:5:2). Plates were processed with iodine vapour (Dao et al., 2011:7).

The quantum-chemical calculations were made with using of Gaussian 09w (Revision D.01) program by method of density functional theory (DFT). B3LYP hybrid functional with 6-31+G(d,p).basic set was used. Effect of solvent was considered within the Polarized Continuum Model (PCM).

General procedure of the receiving of chalcones 1-4.

To 40% sodium hydroxide solution at stirring and a room temperature was dropped the substituted acetophenone solution and aromatic aldehyde in ethanol. In process of aldehyde addition the reactionary mixture had yellow colour. The reactionary mixture was kept at the room temperature for 62-95 h. Then the reactionary mixture was acidified with the diluted hydrochloric acid to neutral medium. The mixture was kept for night in refrigerator. The dropped out light brown powder was filtered, dried and recrystallized from benzene (Hsieh et al., 2000:8).

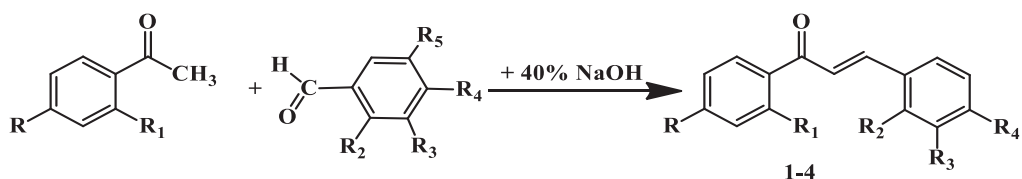
General procedure of the receiving of substituted pyrazolines 5, 6.

To substituted chalcone in ethanol was added an excess of hydrazine hydrate. Mixture was heated at temperature 70-80°C for 4 h, then cooled and diluted in 50 ml of water. A dropped out residue was filtered, washed with water and recrystallized from ethanol.

General procedure of the receiving of flavanones 7,8.

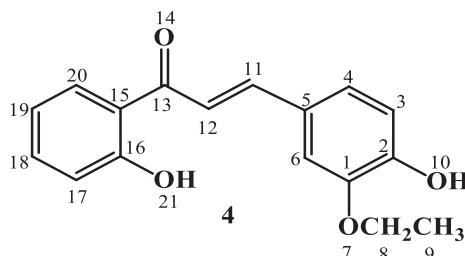
The reactionary mixture from 0.001 mol of substituted chalcone and catalytic amount of triethylamine in 15 ml of ethanol (95%) was heated with backflow condenser for 8 h. The dropped out residue was filtered. It was dried at a room temperature.

**Results and discussion.** Some chalcones, pyrazoles, flavones were previously synthesized, and their anti-inflammatory activity was investigated. In this paper, a part of these compounds was again synthesized to perform the new biological studies on their antiradical activity. Our received results are able to expand the field of their practical application and to study “structure - biological activity” correlation Chalcones (1-4) were received with a following reaction.



- R = HO; R<sub>1</sub> = H; R<sub>2</sub> = R<sub>4</sub> = CH<sub>3</sub>O; R<sub>3</sub> = H (1).  
 R = HO; R<sub>1</sub> = HO; R<sub>2</sub> = R<sub>3</sub> = H; R<sub>4</sub> = CH<sub>3</sub>O (2).  
 R = H; R<sub>1</sub> = HO; R<sub>2</sub> = R<sub>3</sub> = H; R<sub>4</sub> = HO (3).  
 R = H; R<sub>1</sub> = HO; R<sub>2</sub> = H; R<sub>3</sub> = C<sub>2</sub>H<sub>5</sub>O; R<sub>4</sub> = HO (4).

The structure of the synthesized chalcones (1-4) was proved with IR- and <sup>1</sup>H, <sup>13</sup>C NMR spectroscopy IR spectrum of chalcones (1-4) demonstrates intense absorption bands at 1595-1582 cm<sup>-1</sup> correspond to vibrations of C=C bond attached to C=O. <sup>1</sup>H NMR spectrum of compound of (4) shows the strong pole high-intensity triplet signal with a chemical shift (1.33 ppm, <sup>3</sup>J 6.9 Hz) and intensity of 3H belonging to protons of CH<sub>3</sub><sup>9</sup> methyl group. Quadruplet signal (4.11 ppm, <sup>3</sup>J 6.9 Hz) with 2H belong to CH<sub>2</sub><sup>8</sup> methylene group. In a low pole area of spectrum (6.83 ppm, <sup>3</sup>J 8.2 Hz) with intensity 1H were resonated protons of aromatic H<sup>17</sup> system with a doublet. A next aromatic proton H<sup>18</sup> signaled at 7.27 ppm as a doublet of doublets with integrated intensity of 1H (<sup>3</sup>J 8.2, 1.8 Hz). Proton H<sup>3</sup> exhibited at 6.93 ppm as a triplet with intensity 1H (<sup>3</sup>J 8.2 Hz). Other aromatic protons of H<sup>4,6</sup> and H<sup>19,20</sup> gave multiplet signals at 7.50 and 7.75 ppm according to an integrated intensity 2H each. Protons at sp<sup>2</sup>- hybridized carbon H<sup>11</sup> and H<sup>12</sup> atoms give doublet signals at 8.19 и 6.97 ppm with intensity 1H with an identical spin-spin coupling constant (<sup>3</sup>J 7.8 Hz).



<sup>13</sup>C NMR spectrum of the studied compound (4) in the strong pole area of spectrum demonstrates the signals belonging to carbon atoms of ethoxy group at 15.26 (C<sup>9</sup>) and 64.82 (C<sup>8</sup>) ppm. The low pole area shows other signals of <sup>13</sup>C nuclei of the studied molecule. Atoms of benzene nucleus of the ethoxy phenyl radical resonated at 114.1 (C<sup>4</sup>), 118.45 (C<sup>3</sup>), 126.16 (C<sup>5</sup>), 136.67 (C<sup>6</sup>), 147.77 (C<sup>1</sup>) and 153.13 (C<sup>2</sup>) ppm.

Carbon atoms of other benzene nucleus resonated at 116.37 (C<sup>17</sup>), 119.36 (C<sup>20</sup>), 121.14 (C<sup>15</sup>), 125.35 (C<sup>18</sup>), 146.59 (C<sup>19</sup>) and 162.59 (C<sup>16</sup>) ppm. Signals at 118.06 and 131.28 ppm maybe belong to sp<sup>2</sup>- hybridized C<sup>11</sup> and C<sup>12</sup> atoms. The lowest pole signal at 194.17 ppm belongs to C<sup>13</sup>carbonyl atom.

The structure of compound (4) was confirmed with methods of two-dimensional NMR spectroscopy, (<sup>1</sup>H-<sup>1</sup>H) COSY and (<sup>1</sup>H-<sup>13</sup>C) HMQC to establish the homo- and heteronuclear spin-spin interactions. The observed correlations in molecule of (4) are presented in Figure 1. Spectra of <sup>1</sup>H-<sup>1</sup>H COSY of compound

(4) demonstrate the spin-spin correlations through three proton bonds of next methine groups of two aromatic  $H^{17}-H^{18}$ ,  $H^{19}-H^{20}$ ,  $H^3-H^4$  systems and hydrogen at double bond of  $H^{11}-H^{12}$  atoms. All simple proton interactions with carbon atoms through one bond were determined with  $^1H-^{13}C$  HMQC spectroscopy (Hamdi et al., 2011:8).

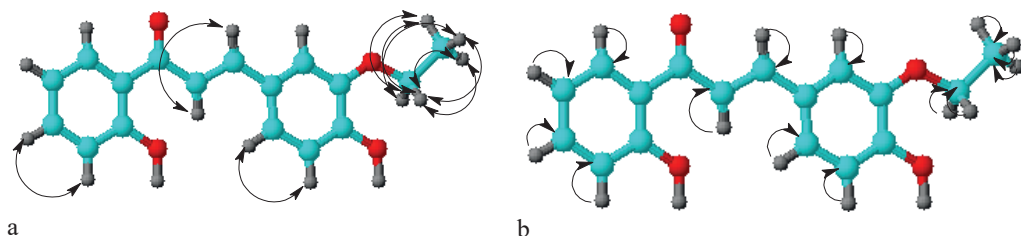
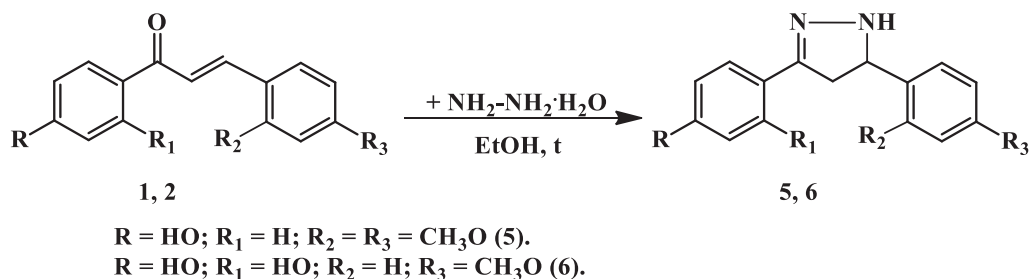


Figure 1: Scheme of correlations in COSY (a) and HMQC (b) spectra of compound 4

Further on the basis of the received chalcones 1, 2, some pyrazole derivatives 5, 6 were received for the biological studies.

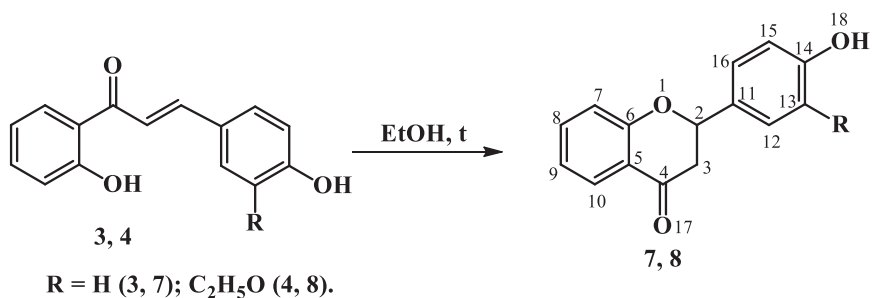


The structure of compounds 5, 6 was determined with methods of the IR- and NMR- spectroscopy. Thus, IR spectra of pyrazolines 5, 6 demonstrate an average intensity strip of  $C=N$ -group of a pyrazoline nucleus at  $1601-1605 \text{ cm}^{-1}$ .

$^1H$  NMR spectrum of 4-[5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-3-yl]benz-1,3-diol (6) shows signals of methylene ( $\delta(H_{ax})=2.84 \text{ ppm}$ ,  $\delta(H_{eq})=3.43 \text{ ppm}$ ) and methine (4.68 ppm) groups of a five-membered nitrogen cycle. The three-proton signal with chemical shift (3.70 ppm) could be attributed to protons of the methoxy substituent of a benzene ring. The low pole area of spectrum (6.27-7.32 ppm) demonstrates the resonating CH-groups of aromatic systems (Litvinenko et al.,1995:8).

In  $^{13}C$  NMR spectrum of compound 6 the secondary and tertiary carbon atoms of a diazocyclic system give the signals at 41.59 and 61.86 ppm respectively. The signal of methoxy group is observed at 55.62 ppm. The united carbon atoms  $C^{14,16}$  and  $C^{13,17}$  of a methoxyphenyl radical resonated at 114.35 and 128.39 ppm

respectively. Signals with the chemical shifts at 102.92, 107.50 and 129.40 ppm could be attributed to methine carbon atoms C<sup>8</sup>, C<sup>10</sup> and C<sup>11</sup> of an aromatic ring. Quarternary carbon atoms give signals at 109.44 (C<sup>6</sup>), 134.76 (C<sup>12</sup>), 153.87 (C<sup>3</sup>), 159.09 (C<sup>9</sup>), 159.74 (C<sup>15</sup>) and 162.10 (C<sup>7</sup>) ppm. Flavonones 7, 8 were developed from the received the 2-hydroxyl chalcones 3, 4 for further studies (Miranda et al., 2000:8).



<sup>1</sup>H NMR spectrum of flavonone 7 demonstrates the strong pole area of spectrum (2.73 ppm) of a doublet of doublets with intensity 1H (<sup>2</sup>J 16.7 Hz and <sup>3</sup>J 3.2 Hz) of an axial proton of methylene group in the condensed system H<sup>3ax</sup>. An equatorial methylene proton of the six-membered cycle H<sup>3eq</sup> has the spin-spin interactions through 2 and 3 bonds. This proton is in a low pole area in comparison with an axial atom (3.18 ppm) of a doublet of doublets with 1H (<sup>2</sup>J 16.7 Hz and <sup>3</sup>J 12.8 Hz). Near methylene proton H<sup>2</sup> resonates a doublet of doublets at 5.48 ppm with the integrated intensity 1H (<sup>3</sup>J 12.8, 2.8 Hz).

The equivalent protons of aromatic system H<sup>13,15</sup> and H<sup>12,16</sup>, having near hydrogen atoms, which are able to split spectrum, were shown with doublet signals at 6.77 (<sup>3</sup>J 8.2 Hz) and 7.30 (<sup>3</sup>J 8.3 Hz) ppm with intensity 2H. The proton H<sup>11</sup> of the studied aromatic cycle, which having no near hydrogen atoms, signaled by a singlet at 6.71 ppm with intensity 1H. Nonsymmetrical aromatic protons H<sup>7,9</sup> were observed as multiplet signal at 7.00-7.05 ppm with the integrated intensity 2H. Other protons H<sup>8</sup> and H<sup>10</sup> were shown as a triplet at 7.52 ppm (<sup>3</sup>J 8.2 Hz) and a doublet at 7.75 ppm (<sup>3</sup>J 7.9 Hz) with the integrated intensity 1H each. The hydroxyl protons OH<sup>18</sup> were observed as a broadened singlet in the lowest pole area of spectrum at 9.48 ppm.

In <sup>13</sup>C NMR spectrum of compound 7 the methylene and methine signals of heterocycle were at 43.94 (C<sup>3</sup>) and 79.40 (C<sup>2</sup>) ppm respectively. Signals with the chemical shifts at 115.82 (C<sup>13</sup>), 115.92 (C<sup>15</sup>), 118.76 (C<sup>7,9</sup>), 121.19 (C<sup>5</sup>), 128.54 (C<sup>12</sup>), 128.91 (C<sup>16</sup>), 129.69 (C<sup>11</sup>), 136.80 (C<sup>8</sup>), 158.19 (C<sup>14</sup>) and 161.77 (C<sup>6</sup>) ppm belong to carbon nuclei of two aromatic rings. In the lowest area of spectrum at 192.40 ppm resonated a carbonyl carbon atom C<sup>4</sup>.

The structure of compound 7 was confirmed with methods of two-dimensional NMR spectroscopy, ( $^1\text{H}$ - $^1\text{H}$ ) COSY and ( $^1\text{H}$ - $^{13}\text{C}$ ) HMQC to establish the homo- and heteronuclear spin-spin interactions. The observed correlations in molecule are presented in Figure 2. Spectra of  $^1\text{H}$ - $^1\text{H}$  COSY of compound 7 demonstrate the spin-spin correlations through three proton bonds of next methine groups  $\text{H}^{7,9}$ - $\text{H}^{8,10}$ ,  $\text{H}^{12,15}$ - $\text{H}^{13,16}$  and methine- methylene groups  $\text{H}^2$ - $\text{H}^3$ . The heteronuclear proton interactions with carbon atoms through one bond were determined with  $^1\text{H}$ - $^{13}\text{C}$  HMQC for all couples in bond:  $\text{H}^2$ - $\text{C}^2$ ,  $\text{H}^{12,16}$ - $\text{C}^{12,16}$ ,  $\text{H}^{13}$ - $\text{C}^{13}$ ,  $\text{H}^{15}$ - $\text{C}^{15}$ ,  $\text{H}^{7,9}$ - $\text{C}^{7,9}$ ,  $\text{H}^8$ - $\text{C}^8$  и  $\text{H}^{10}$ - $\text{C}^{10}$ .

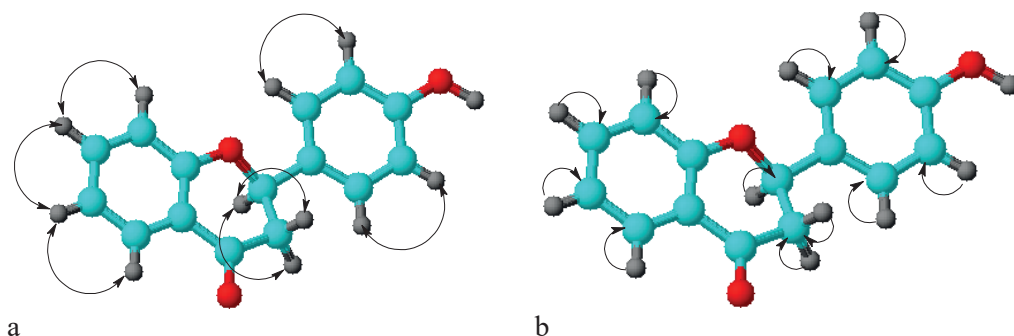


Figure 2: Scheme of correlations in COSY (a) and HMQC (b) spectra of compound 7

In order to research the synthesized compounds 2-8 the screening of antiradical activity based on interaction of compounds with the stable chromogen radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).

The DPPH methanol solution (100  $\mu\text{M}$ ) was used to evaluate the antiradical activity of the studied samples in the test with the DPPH radical. In order to select substances with the antiradical activity, 2 ml of the DPPH methanol solution (100  $\mu\text{M}$ ) was mixed with 20  $\mu\text{L}$  of the studied object dissolved in methanol concentration 5 mM. Thus, the final concentration of the studied substance in reactionary mixture made 50  $\mu\text{M}$ . The reduction in optical density at 515 nm was measured in 10 min after addition of the studied substance solution to the DPPH radical solution. The substances, which are able to reduce optical density more 50%, were tested for interaction with the DPPH radical in the final concentration of the studied substances (50, 25, 20, 15, 10, 5 and 2.5  $\mu\text{M}$ ). Then concentration of the studied substance reducing an optical density by 50% -  $\text{IC}_{50}(\text{DPPH})$  was determined. Research results of the biological activity of chalcones 2-4, pyrazolines 5, 6 and flavonones 7, 8 are presented in Table 1.

Table 1: The optical density values of the DPPH radical solution (100  $\mu\text{M}$ ) after 10 min incubation with the studied substances 2-8 in final concentration 50  $\mu\text{M}$

No.	Compound	The optical density
1	2	0.856
2	3	0.941
3	4	0.188
4	5	0.851
5	6	0.120
6	7	0.911
7	8	1.005
8	Control (DPPH solution without the studied substance)	0.852

Table 1 demonstrates that compounds 4 and 6 reduce the optical density of the initial DPPH radical solution more 50%. Thus they are perspective for further researches. Other compounds did not have a high antiradical activity in this test system.

The second series of experiments studied the ability of compounds 4 and 6 in various concentrations (2.5 - 50  $\mu\text{M}$ ) to interact with the DPPH radical. Research results of antiradical activity of compounds 4 and 6 are shown in Table 2.

Table 2: The optical density values of the DPPH radical solution (100  $\mu\text{M}$ ) after 10 min incubation with the substances 4 and 6 in final concentration in reaction mixture (50, 25, 20, 15, 10, 5 and 2.5  $\mu\text{M}$ )

No.	Final concentration 4 and 6 in reaction mixture, $\mu\text{M}$	The optical density	
		compound 4	compound 6
1.	50	0.230	0.055
2.	25	0.794	0.351
3.	20	0.784	0.444
4.	15	0.835	0.557
5.	10	0.884	0.658
6.	5	0.951	0.763
7.	2.5	0.985	0.811
8.	Control (DPPH solution without the studied substance)	1.038	0.907

Concentrations of substances 4 and 6 which are able to reduce by 50% the optical density of the DPPH radical solution (100  $\mu\text{M}$ ) were defined with the plotting calibration curves shown in Figure 3.  $\text{IC}_{50}(\text{DPPH})$  was equal 38.4  $\mu\text{M}$  for 4.  $\text{IC}_{50}(\text{DPPH})$  made 19.8  $\mu\text{M}$  for 6.

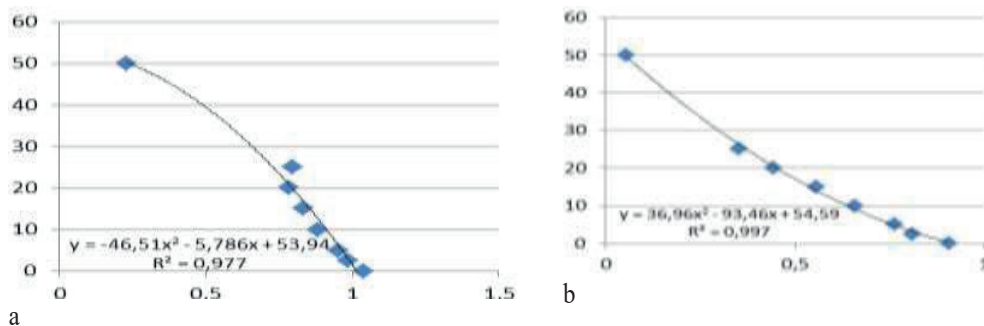
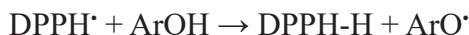


Figure 3: Dependences of the optical density of the DPPH radical solution on concentrations 4 (a) and 6 (b)

Literary data (Nurkenov, et al., 2019:8) reported that  $IC_{50}$ (DPPH) ( $\mu$ M) for ascorbic acid – 27, glutathione – 49, hydroquinone – 27, trolox – 28,  $\alpha$ -tocopherol – 28 and quercetin - 8. Thus, the activity of samples 4 and 6 is comparable to activity of the known antioxidants.

The received (Sivakumar et al., 2011:8) views reported that interaction of compounds with the DPPH radical, which having the phenolic hydroxyl groups, was obtained with the homolytic decomposition of O-H bond, i.e. through intermediate formation of a phenoxy radical:



This stage is limiting (Plattner et al., 2014:8) therefore the reactionary ability of a potential antioxidant ArOH with the DPPH radical has to be determined with the homolytic O-H bond dissociation energy:



In order to verify this hypothesis the calculations were made for change of enthalpy  $\Delta H_d$  as a measure of O-H bond dissociation energy in compounds 2-8 with a quantum-chemical method of density functional B3LYP/6-31+G(d,p) including effect of solvent (methanol) within the PCM model (Satyanarayana et al., 2004:8). As a result of geometry optimization the inferior limits on a potential energy surface (PES) of compounds 2-8, and on all possible phenoxy radicals formed of these molecules were found out. It should be pointed out in molecules 2, 3, 4, 6 containing two phenolic hydroxyls, the radicals  $ArO\cdot$  are formed at O-H bond dissociation in position 4 of a relevant benzene ring (Tiwari et al., 2012:8). Energy of these stable radicals was a basis to calculate  $\Delta H_d$  values of compounds 2, 3, 4, 6. The deep inferior limits on PES correspond to structures



with possible forming of the intramolecular hydrogen bonds of  $\text{OH}\cdots\text{N}$  or  $\text{OH}\cdots\text{O}$  (see Fig. 4). The calculation results demonstrate that the geometrical structure of radicals  $\text{ArO}\cdot$  differs moderately from a structure of the relevant initial molecules (Volkov et al., 2009:8).



Figure 4: Structure of compounds 6 (a) and 8 (b) in methanol solution by geometry optimization with using the method of density functional.

The O-H bond dissociation energies, which evaluated as enthalpies  $\Delta H_d$  for the studied compounds, made 363.7 (2), 340.4 (3), 331.1 (4), 340.4 (5), 321.8 (6), 347.3 (7), 333.8 kJ/mol (8) (see Figure 5).

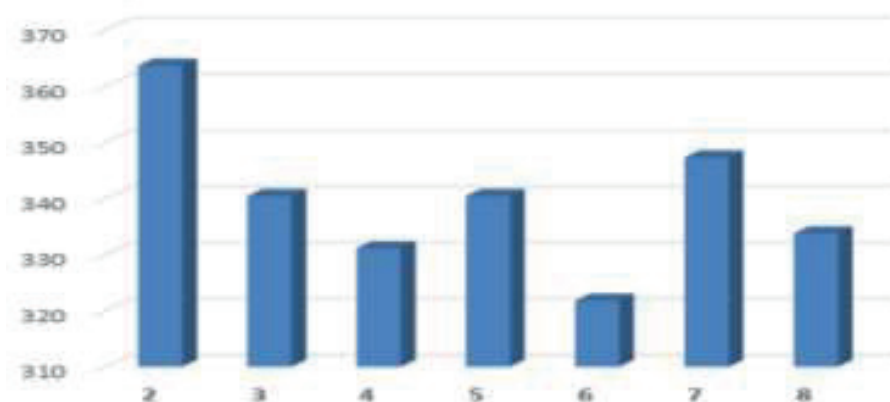


Figure 5: Heats of formation of phenoxy radicals  $\Delta H_d$  (kJ/mol) from compounds 2-8 according to the quantum-chemical calculations data.

**Conclusion.** Phenoxy radicals are easily formed of compounds 4 and 6 which coordinated with their observed antiradical activity (Tab. 1). Substituted 4.5-dihydropyrazole 6, showing the high antiradical properties among the studied chalcones and their derivatives, has a value  $\Delta H_d$  20-40 kJ/mol less than inactive compounds 2, 3, 5, 7. Thus, a high tendency to the homolytic O-H bond dissociation might be one of the basic reasons of the observed antiradical activity of compounds 4 and 6. In addition, the change of an enthalpy  $\Delta H_d$  exceeds by 2.7 kJ/mol for an inactive flavonone 8 then formation energy of a phenoxy radical from very active chalcone 4. It seems that it is explained with the steric effect of ethoxy group, blocking an interaction of a located hydroxyl (Fig. 4 b) close with the DPPH radical. Its specific solvation with methanol, which is not within the PCM model, might be other possible reason of absence of antiradical properties in compound 8.

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