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Synthesis, Structure, and Anti-Inflammatory Activity of Functionally Substituted Chalcones and Their Derivatives

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Abstract—Functionally substituted chalcones, pyrazolines, and flavonones have been synthesized. Their structure has been studied by means of ^{1}H and ^{13}C NMR spectroscopy, including COSY and HMQC experiments. Anti-inflammatory activity of the synthesized chalcones, pyrazolines, and flavonones has been evaluated.

Keywords: substituted aromatic aldehydes, chalcone, pyrazoline, flavonone, cytokine, transcription factor NF-κB

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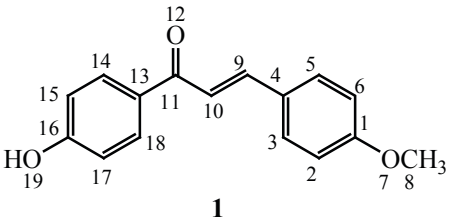
Chalcones are of great interest because of easy synthesis, high pharmacological activity, and possibility to be used as synthons for obtaining many biologically active heterocyclic compounds, in particular pyrazolines and flavones [1]. The compounds with chalcone fragment exhibit significant antitumor, antibacterial, antifungal, antiviral, antimalarial, antihyperglycemic, anti-inflammatory, and immunomodulating activity and possess chemoprotective and antioxidant properties [2–12]. Moreover, certain chalcones can secure blood capillaries [6]. Thus, synthesis of novel chalcones and nitrogen-containing heterocyclic compounds based on them is an important issue.

Herein we studied the reactions of hydroxyacetophenones with substituted aromatic aldehydes in the presence of aqueous-alcoholic alkali solution (the Claisen–Schmidt condensation). The reaction was carried out at equimolar ratio of the components in the presence of 40% solution of NaOH at room temperature for 62–85 h (Scheme 1). The reaction progress was monitored using thin-layer chromatography. The obtained chalcones **1**–**6** were colored powders soluble in benzene and alcohols.

The structures of the prepared chalcones **1**–**6** were confirmed using IR as well as ^{1}H and ^{13}C NMR spectroscopy. The IR spectra of compounds **1**–**6** contained strong absorption bands in the 1595–1582 cm^{-1} region, corresponding to the vibrations of C=C bond conjugated with the carbonyl group.

The ^{1}H NMR spectrum of compound **1** in deuterated DMSO contained a strong signal at 3.76 ppm (intensity of 3H) corresponding to protons of the methyl group OCH_3[8]. The equivalent protons of the methoxyphenyl fragment $H^{2,6}$ and $H^{3,5}$ resonated as doublets at 6.95 (2H, ^{3}J = 8.6 Hz) and 7.77 ppm (2H, ^{3}J = 8.6 Hz), respectively. The protons at the double bond, H^{9} and H^{10}, appeared as doublet signals at 7.74 and 7.62 ppm (^{3}J = 16.2 Hz), indicating *trans*-configuration of the C=C bond. The equivalent CH protons of another aromatic system appeared as doublet signals at 6.86 ($H^{15,17}$, ^{3}J = 8.9 Hz) and 8.03 ppm ($H^{14,18}$, ^{3}J = 8.9 Hz). The broadened singlet signal at 10.39 ppm indicated the presence of the phenolic OH-group.



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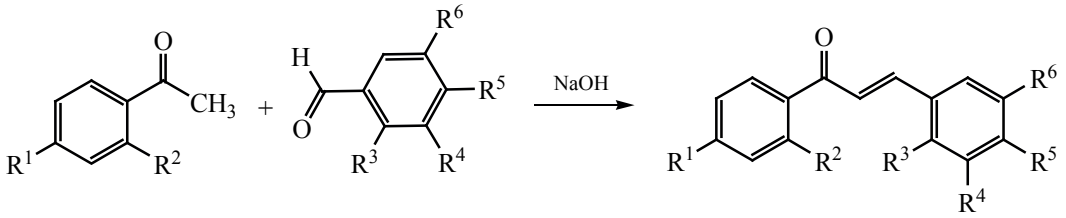
Scheme 1.



1–6

R^1 = OH, R^2 = R^3 = R^4 = R^6 = H, R^5 = OMe (**1**); R^1 = R^4 = R^5 = R^6 = H, R^2 = R^3 = OH (**2**); R^1 = R^2 = OH, R^3 = R^4 = R^6 = H, R^5 = OMe (**3**); R^1 = R^3 = R^4 = R^6 = H, R^2 = R^5 = OH (**4**); R^1 = R^3 = R^6 = H, R^2 = R^5 = OH, R^4 = OEt (**5**); R^1 = R^6 = Br, R^2 = R^4 = R^5 = H, R^3 = OH (**6**).

The ^{13}C NMR spectrum of compound **1** contained the signal of methoxy group at 55.83 ppm. The signals at 114.87 ($C^{2,6}$), 115.88 ($C^{15,17}$), 131.05 ($C^{3,5}$), and 131.57 ppm ($C^{14,18}$) corresponded to the carbon atoms of aromatic systems. Quaternary carbon atoms appeared at 161.62 (C^1), 128.04 (C^4), 129.84 (C^{13}), and 162.61 (C^{16}) ppm. The signals at 120.08 and 143.21 ppm could be assigned to the C^9 and C^{10} carbon atoms involved in the multiple bond formation. The signal in the weakest field (187.57 ppm) corresponded to the C^{11} atom of the carbonyl group.

The structure of compound **1** was also confirmed by two-dimensional COSY ^1H–^1H and HMQC ^1H–^{13}C NMR methods revealing the homo- and heteronuclear spin-spin correlations. The ^1H–^1H COSY spectra of compound **1** contained the spin-spin correlations of aromatic protons and olefinic protons H^9 and H^{10} via three bonds (Fig. 1a). Simple interactions between the protons and carbon atoms were found using ^1H–^{13}C HMQC spectroscopy (Fig. 1b).

Cyclocondensation reaction of hydrazines with α,β-unsaturated ketones is an important synthetic route to obtain 1,2-azoles. Several pyrazole derivatives exhibit properties of analgesics and thrombocyte aggregation inhibitors [13], possess strong antibacterial [14] and anesthetic [15] action.

We investigated the reactions of the prepared chalcones **1**–**6** with hydrazine hydrate to further functionalize the obtained compounds. It was observed that refluxing the chalcones with hydrazine hydrate in ethanol resulted in intramolecular cyclocondensation of intermediate hydrazine with the formation of the corresponding pyrazole derivatives **7**–**11** (Scheme 2).

Structures of compounds **7**–**11** were confirmed using IR and NMR spectroscopy. For example, IR spectra of pyrazolines **7**–**11** revealed distinct absorption band corresponding to the C=N bond of pyrazole cycle at 1601–1605 cm^{-1}. ^1H NMR spectrum of 4-[5-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]phenol **7** contained four groups of signals in weak field corresponded to protons of 4-hydroxy and 4-methoxyphenyl fragments. Two doublets at 7.40 and 7.23 ppm corresponded to *ortho* and *meta*-protons of 4-hydroxyphenyl fragment, two doublets at 6.84 and 6.72 ppm corresponded to *ortho* and *meta*-protons 4-methoxyphenyl fragment. A strong singlet at 3.67 ppm could be assigned to protons of methoxy group. Another group of signals (triplet at 4.63–4.68 ppm) corresponded to methine proton of pyrazoline fragment. Methylene protons of that fragment resonated at 2.65–2.72 ppm as two doublets. A weak signal at 9.67 ppm corresponded to the NH-proton of the pyrazoline fragment.

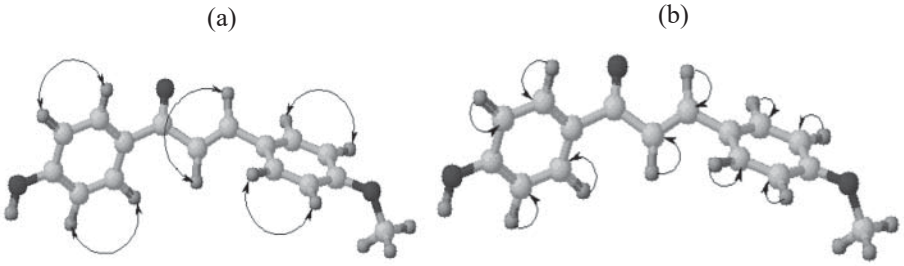
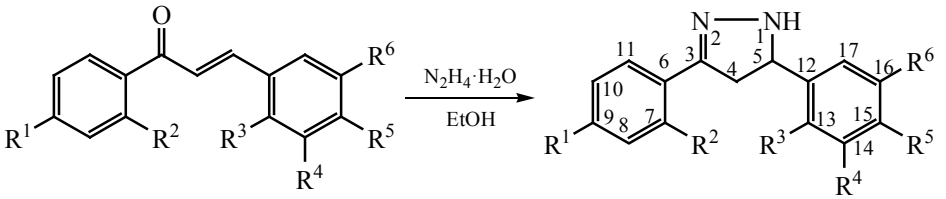


Fig. 1. Main correlations in COSY (a) and HMQC (b) spectra of compound **1**.

Scheme 2.



1–6 **7–11**

R^1 = OH, R^2 = R^3 = R^4 = R^6 = H, R^5 = OMe (**7**); R^1 = R^4 = R^5 = R^6 = H, R^2 = R^3 = OH (**8**); R^1 = R^2 = OH, R^3 = R^4 = R^6 = H, R^5 = OMe (**9**); R^1 = R^3 = R^4 = R^6 = H, R^2 = R^5 = OH (**10**); R^1 = R^3 = R^6 = H, R^2 = R^5 = OH, R^4 = OEt (**11**).

Certain processes of chalcones and flavonones biogenesis in plants are similar. Therefore, combination of structural features of these compounds in a single molecule is a promising approach to obtain highly efficient bioactive compounds [16, 17]. So, we prepared flavonones **12**–**15** from the obtained 2-hydroxychalcones in ethanol medium in the presence of catalytic amount of triethylamine. Prolonged refluxing in 95% ethanol led to the formation of flavonones **12**–**15** (Scheme 3). It was shown that transformation of chalcones into flavonones was catalyzed by water molecules.

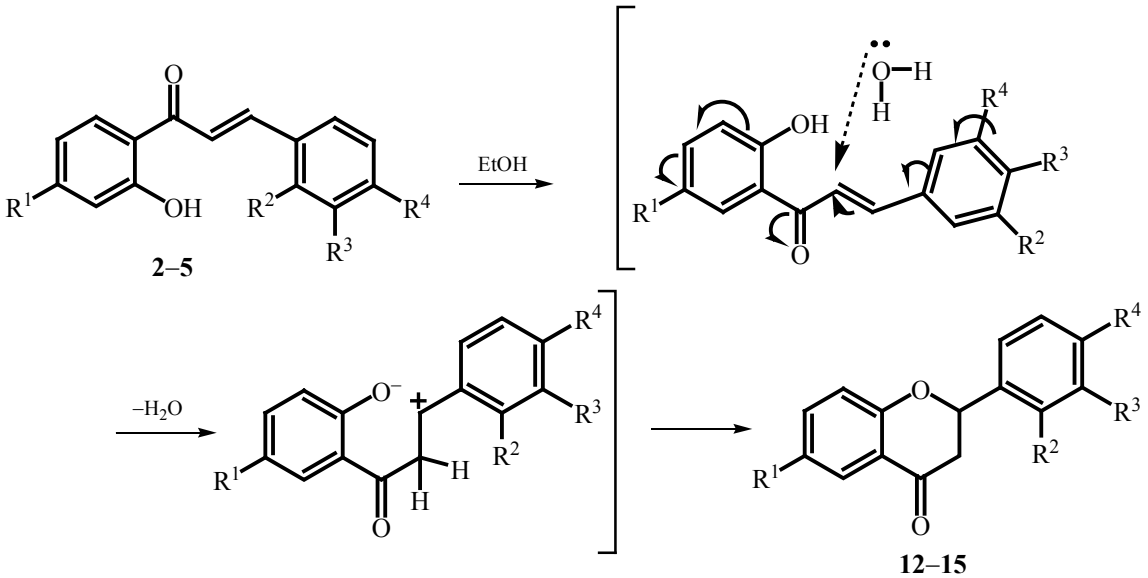
The structures of flavonones **12**–**15** were confirmed using IR as well as ^{1}H and ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum of compound **15** contained a triplet at 1.29 ppm and a multiplet at 3.98–4.03 ppm corresponding to protons of ethoxy group at the C^{20} and C^{19} atoms. The protons of methylene and CH groups of the fused rings appeared at 5.47 (H^2), 2.69–3.31 (H^3), 7.06 (H^7), 7.55 (H^8), 7.76 (H^9), and 7.04 ppm (H^{10}). The protons of CH groups of the phenyl fragments resonated at 6.77–6.90 ppm. A signal in the weakest field at 9.00 ppm corresponded to the H^{21} atom of the hydroxyl group.

The assignment of the ^{13}C NMR signals was carried out using the DEPT method. The assignment of the ^{1}H NMR signals was made using two-dimensional COSY and TOCSY NMR spectroscopy (Fig. 2).

Anti-inflammatory and cytotoxic activity of the prepared chalcones **1**, **3**–**5**, pyrazolines **8**, **9**, and flavones **12**, **14**, and **15** was estimated on the cultures of human monocytic cell lines MonoMac-6 and THP-1Blue. The results are given in the table.

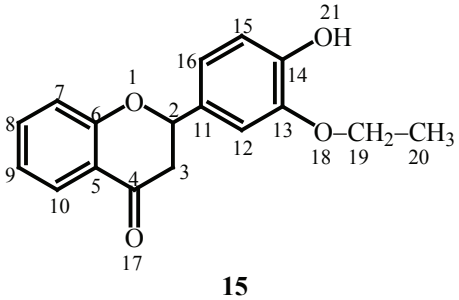
It was figured out that chalcone **5** and flavone **15** were cytotoxic against monocytic MonoMac-6 cells, so it was

Scheme 3.



R^1 = R^3 = R^4 = H, R^2 = OH (**12**); R^1 = OH, R^2 = R^3 = H, R^4 = OMe (**13**); R^1 = R^2 = R^3 = H, R^4 = OH (**14**); R^1 = R^2 = H, R^3 = OEt, R^4 = OH (**15**).

impossible to correctly estimate their anti-inflammatory potential for that culture. Pyrazoline **9** and flavone **14** inhibited the production of anti-inflammatory cytokines interleukin-6 (IL6) and tumor necrosis factor (TNF). However, those compounds possibly should not be considered as promising ones because of their low activity (IC_{50} > 30 μM).



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Chalcones **1**, **3**, and **4**, pyrazoline **8**, and flavone **12** inhibited the production of tumor necrosis factor and/or interleukin-6. The mechanism of inhibition of tumor necrosis factor and interleukin-6 production by those compounds obviously was independent of the transcriptional activity of NF-κB. The reason was the fact that

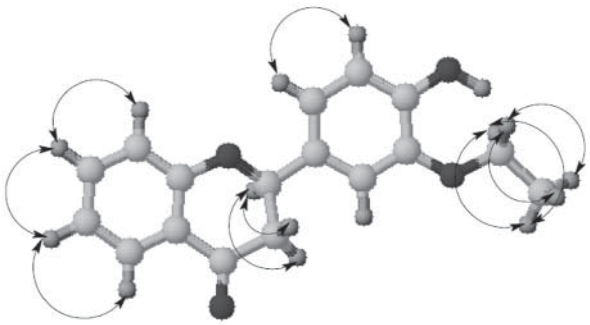

Fig. 2. Main correlations in COSY ^1H–^1H and TOCSY ^1H–^1H spectra of compound **15**.

inhibition of the NF-κB activity which was estimated using the level of alkaline phosphatase production in THP-1Blue cells was very low (compound **3** and **4**) or zero (compounds **1**, **8**, and **12**). Those compounds and their analogs could recommended for further investigation of anti-inflammatory activity, because they did not exhibit (**1**, **12**) or practically did not exhibit (**3**, **4**, **8**) cytotoxic activity at the concentration range with found IC_{50} values in the inhibition of cytokine production test.

Anti-inflammatory activity (*in vitro*), cytotoxicity, physical and chemical properties of the investigated chalcones, pyrazolines, and flavonones

Comp. no.	IC_{50}, μM					*S*, Å^2	lg*P*	E_h, kcal/mol
	MonoMac-6 cells			THP-1Blue cells				
	TNF[a]	IL6[a]	toxicity	alkali phosphatase	toxicity			
				Chalcones				
1	9.9 (A)	24.2 (A)	–[b]	–[b]	82.1	430.9	3.14	–11.97
3	15.6 (A)	18.5 (A)	75.0	51.2	36.5	430.5	2.85	–17.49
4	10.9 (A)	19.7 (A)	69.5	53.0	71.2	368.4	3.11	–16.12
5	17.4 (–)	–[b] (–)	32.1	48.4	27.8	442.5	3.20	–15.62
				Pyrazolines				
8	–[b] (N)	9.6 (A)	>60	–[b]	–[b]	311.8	2.90	–12.95
9	52.0 (N)	33.8 (N)	–[b]	–[b]	–[b]	406.1	2.64	–18.76
				Flavonones				
12	35.0 (N)	15.5 (A)	–[b]	–[b]	–[b]	308.9	2.56	–8.78
14	51.0 (N)	50.0 (N)	–[b]	–[b]	–[b]	334.7	2.56	–10.86
15	24.5 (–)	9.0 (–)	35.5	>50	34.1	412.1	2.65	–10.43

[a] No inhibition of production or cytotoxicity at concentrations <100 μM.

[b] The compounds considered active (A, IC_{50}<30 μM) or conditionally inactive (N, IC_{50}>30 μM) during classification analysis are marked in parentheses.

To corelate the biological activity of the compounds with their structure (SAR analysis), we calculated several physical and chemical parameters of the molecules using the additive schemes implemented in HyperChem software. Molecular surface (*S*), logarithm of distribution coefficient in octanol–water system (lipophilicity log *P*), hydration energy (E_h) (see the table) as well as molecular volume (*V*) and polarizability (α) were thus obtained. The considered compounds were divided into two groups, active (A) and inactive (or relative inactive, N) according to the values of two types of biological activity towards the MonoMac-6 cells to perform their linear discriminant analysis (STATISTICA 8 software) (see the table). A compound was assigned to the A group if its IC_{50} value was below 30 μM. Compounds **5** and **15** exhibited toxicity against MonoMac-6 cells, so those two compounds were not used in the linear discriminant analysis. For other chalcones, pyrazolines, and flavonones, linear discriminant analysis allowed elucidation of the most important parameters, according to which a compound could be classified into the A and N groups by each of the considered types of activity (TNF and IL6). Despite the low number of compounds, statistically significant coefficients of classification functions ($p < 0.05$) were obtained.

$$\mathrm{TNF(A)} = -292.45 + 0.333S + 147.11\log P, \quad (1)$$

$$\mathrm{TNF(N)} = -224.96 + 0.287S + 129.85\log P. \quad (2)$$

According to the principles of linear discriminant analysis, the meaning of the obtained classification functions (1) and (2) was as follows. The *S* and log *P* values of a tested compound were substituted into Eqs. (1) and (2), and the values of both functions were calculated. Then if TNF(A)> TNF(N), the compound was assigned to the "active" group with respect to the tumor necrosis factor, otherwise it was considered "inactive". The given equations correctly classified the 9 considered compounds with the experimentally available tumor necrosis factor production values into the N or A groups (see the table). According to Eqs. (1) and (2), the increase in either molecular surface or lipophilicity resulted in the increase in the activity estimated by inhibition of tumor necrosis factor production, because the corresponding coefficients in Eq. (1) were higher than those in Eq. (2).

Similarly, the 9 compounds were correctly classified by interleukin-6 production using classification functions (3) and (4).

$$\mathrm{IL6(A)} = -124.74 + 1.012E_h + 89.95\log P, \quad (3)$$

$$\mathrm{IL6(N)} = -99.28 + 0.535E_h + 77.94\log P. \quad (4)$$

Physical and chemical parameters *V* and α were statistically insignificant for the compounds classification as revealed during optimization of functions (1)–(4). The lipophilicity value as well as hydration energy E_h were significant for the activity estimated by inhibition of interleukin-6 production (less negative E_h values increased the activity).

Thus, one of the main parameters influencing both types of the biological activity determined on the MonoMac-6 cells was lipophilicity of the investigated chalcones, pyrazolines, and flavonones. It is interesting that the $-\log IC_{50}$(TNF) values of compounds **1**, **3**, **4**, **9**, **12**, and **14** showed satisfactory linear correlation (5) with log *P* values:

$$-\log IC_{50}(\mathrm{TNF}) = 1.327 + 0.948\log P, \quad (5)$$

$$r = 0.95,\ F = 35.7,\ s = 0.117.$$

The obtained results may indicate the significant contribution of bioavailability (e.g. ability to penetrate through cellular membrane) to both types of biological activity determined on the MonoMac-6 cells.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded using a JNN-ECA Jeol 400 spectrometer (399.78 and 100.53 MHz, respectively); DMSO-d_6 was used as solvent. The reactions course and purity of the obtained products were monitored using thin-layer chromatography on Silufol UV-254 plates in isopropanol–benzene–ammonia (10 : 5 : 2) system. The plates were developed with iodine vapor.

Chalcones 1–6 (*general procedure*). A solution of 0.013 mol of substituted acetophenone and 0.013 mol of aromatic aldehyde in 20 mL of ethanol was dropwise added to 20 mL of 40% solution of sodium hydroxide under stirring at room temperature. During the addition of aldehyde, the mixture turned yellow. The reaction mixture was kept at room temperature for 62–95 h, then dilute hydrochloric acid was added to pH = 7, and the mixture was kept overnight at –15°C. The precipitate was filtered off, dried, and recrystallized from benzene.

(*E*)-1-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-prop-2-en-1-one (1). Yield 36%, mp 186–187°C. ^{1}H NMR spectrum, δ, ppm: 3.76 s (3H, H^8), 6.86 d (2H, $H^{15,17}$, ^3J = 8.9 Hz), 6.95 d (2H, $H^{2,6}$, ^3J = 8.6 Hz), 7.62 d (1H, H^{10}, ^3J = 16.2 Hz), 7.74 d (1H, H^9, ^3J = 16.2 Hz), 7.77 d (2H, $H^{3,5}$, ^3J = 8.6 Hz), 8.03 d (2H, $H^{14,18}$, ^3J = 8.9 Hz), 10.39 br. s (1H, OH). ^{13}C NMR spectrum, δ_C, ppm: 55.83 (C^8), 114.87 ($C^{2,6}$), 115.88 ($C^{15,17}$), 120.08

(C^9), 128,04 (C^4), 129.84 (C^{13}), 131.05 ($C^{3,5}$), 131.57 ($C^{14,18}$), 143.21(C^{10}), 161.62 (C^1), 162.61 (C^{16}), 187.57 (C^{11}).

(*E*)-1,3-Bis(2-hydroxyphenyl)prop-2-en-1-one (2). Yield 84%, mp 154–155°C. ^{1}H NMR spectrum, δ, ppm: 6.85 t (H, H^{14}, ^3J = 8.7 Hz), 6.90–6.98 m (3H, $H^{4,6,10}$), 7.26 t (1H, H^{15}, ^3J = 8.2 Hz), 7.51 t (1H, H^5, ^3J = 7.8 Hz), 7.81 d (1H, H^{17}, ^3J = 9.6 Hz), 7.89 d (1H, H^{16}, ^3J = 15.6 Hz), 8.07–8.13 m (2H, $H^{3,10}$). ^{13}C NMR spectrum, δ_C, ppm: 116.75 (C^{14}), 118.04 (C^{10}), 119.87 (C^6), 121.03 (C^4), 121.11 (C^{16}), 121.45 (C^2), 121.83 (C^{12}), 129.55 (C^{17}), 131.08 (C^3), 132.80 (C^{15}), 136.64 (C^5), 140.95 (C^{11}), 158.10 (C^{13}), 194.44 (C^8).

(*E*)-1-(2,4-Dihydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (3). Yield 23.4%, mp 175–176°C. ^{1}H NMR spectrum, δ, ppm: 3.78 s (3H, H^{20}), 6.08 d (1H, H^6, ^4J = 2.3 Hz), 6.26 d. d (1H, $H^4{}_{Ar}$, ^3J = 2.1, 8.9 Hz), 6.97 d (2H, $H^{15,17}$, ^3J = 8.7 Hz), 7.69–7.77 m (2H, $H^{11,12}$), 7.79 d (2H, $H^{14,18}$, ^3J = 8.7 Hz), 8.01 d (1H, H^3, ^3J = 9.2 Hz). ^{13}C NMR spectrum, δ_C, ppm: 55.88 (C^{20}), 110.54 (C^6), 111.51 (C^4), 114.91 (C^2), 114.92 ($C^{15,17}$), 119.52 (C^{11}), 128.06 (C^{13}), 131.21 ($C^{14,18}$), 133.08 (C^{12}), 142.94 (C^3), 161.73 (C^{16}), 166.92 (C^1), 167.30 (C^5), 190.52 (C^8).

(*E*)-1-(2-Hydroxyphenyl)-3-(4-hydroxyphenyl)-prop-2-en-1-one (4). Yield 37%, mp 155–156°C. ^{1}H NMR spectrum, δ, ppm: 6.82 d (2H, $H^{13,17}$, ^3J = 8.7 Hz), 6.94 m (1H, H^4), 6.96 d (1H, H^{11}, ^3J = 11.9 Hz), 7.49 m (1H, H^3), 7.69–7.75 m (2H, $H^{5,6}$), 7.72 d (2H, $H^{14,16}$, ^3J = 8.7 Hz), 8.5 d (1H, H^{10}, ^3J = 7.8 Hz). ^{13}C NMR spectrum, δ_C, ppm: 116.37 (C^{13}), 116.67 (C^{17}), 118.39 (C^{11}), 119.60 (C^4), 121.20 (C^2), 126.17 (C^{12}), 131.05 (C^{10}), 131.87 (C^{14}), 131.94 (C^{16}), 136.53 (C^3), 146.10 ($C^{5,6}$), 161.12 (C^{15}), 162.51 (C^1), 194.13 (C^8).

(*E*)-1-(2-Hydroxyphenyl)-3-(3-ethoxy-4-hydroxyphenyl)prop-2-en-1-one (5). Yield 72%, mp 151–152°C. ^{1}H NMR spectrum, δ, ppm: 1.33 t (3H, H^9, ^3J = 6.9 Hz), 4.11 q (2H, H^8, ^3J = 6.9 Hz), 6.83 d (1H, H^{17}, ^3J = 8.2 Hz), 6.93 t (1H, H^3, ^3J = 8.2 Hz), 6.97 d (1H, H^{12}, ^3J = 7.8 Hz), 7.27 d. d (1H, H^{18}, ^3J = 8.2, 1.8 Hz), 7.50 m (2H, $H^{4,6}$), 7.75 m (2H, $H^{19,20}$), 8.19 d (1H, H^{11}, ^3J = 7.8 Hz). ^{13}C NMR spectrum, δ_C, ppm: 15.26 (C^9), 64.82 (C^8), 114.11 (C^4), 116.39 (C^{17}), 118.06 (C^{11}), 118.45 (C^3), 119.36 (C^{20}), 121.14 (C^{15}), 125.35 (C^{18}), 126.16 (C^5), 131.28 (C^{12}), 136.67 (C^6), 146.59 (C^{19}), 147.77 (C^1), 153.13 (C^2), 162.59 (C^{16}), 194.17 (C^{13}).

(*E*)-1-(4-Bromophenyl)-3-(5-bromo-2-hydroxyphenyl)prop-2-en-1-one (6). Yield 35%, mp 184–185°C. ^{1}H NMR spectrum, δ, ppm: 6.84 d (1H, H^3, ^3J = 9.2 Hz), 7.37 d. d (1H, H^2, ^3J = 8.7, 2.3 Hz), 7.73 d (2H, $H^{15,17}$, ^3J = 7.4 Hz), 7.86–7.96 m (2H, $H^{6,10}$), 8.05 d (2H, $H^{14,18}$, ^3J = 8.3 Hz), 8.11 d (1H, H^9, ^3J = 2.3 Hz). ^{13}C NMR spectrum, δ_C, ppm: 111.40 (C^1), 118.85 (C^3), 121.99 (C^{10}), 124.05 (C^5), 127.85 (C^{16}), 130.85 (C^6), 132.35 ($C^{15,17}$), 134.97 (C^2), 137.07 (C^{13}), 138.47 (C^9), 178.78 (C^{11}).

Substituted pyrazolines 7–11 (*general procedure*). 0.02 mol of hydrazine hydrate was added to 0.002 mol of substituted chalcone in 10 mL of ethanol. The mixture was heated at 70–80°C for 4 h. Then the mixture was cooled to ambient and diluted with 50 mL of water. The precipitate was filtered off, washed with water, and recrystallized from ethanol.

4-[5-(4-Methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]phenol (7). Yield 53%, mp 119–120°C. ^{1}H NMR spectrum, δ, ppm: 2.68 d. d (1H, H^{4ax}, ^2J = 16.4, ^3J = 11.0 Hz), 3.27 d. d (1H, H^{4eq}, ^2J = 16.4, ^3J = 10.5 Hz), 3.67 s (1H, H^{20}), 4.68 t (1H, H^5, ^3J = 10.1 Hz), 6.72 d (2H, $H^{8,10}$, ^3J = 8.7 Hz), 6.84 d (2H, $H^{14,16}$, ^3J = 8.7 Hz), 7.21 d (2H, $H^{13,17}$, ^3J = 8.7 Hz), 7.40 d (2H, $H^{7,11}$, ^3J = 8.2 Hz), 9.67 br. s (1H, OH). ^{13}C NMR spectrum, δ_C, ppm: 41.42 (C^4), 55.55 (C^5), 63.51 (C^{20}), 114.22 ($C^{14,16}$), 115.84 ($C^{8,10}$), 124.92 (C^6), 127.52 ($C^{13,17}$), 128.28 ($C^{7,11}$), 135.51 (C^{12}), 149.71 (C^3), 158.16 (C^{15}), 158.86 (C^9).

2,2'-(4,5-Dihydro-1*H*-pyrazol-3,5-diyl)phenol (8). Yield 72%, mp 124–125°C. ^{1}H NMR spectrum, δ, ppm: 2.88 d. d (1H, H^{4ax}, ^2J = 16.6, ^3J = 10.1 Hz), 3.53 d. d (1H, H^{4eq}, ^2J = 16.6, ^3J = 10.7 Hz), 5.00 t (1H, H^5, ^3J = 10.5 Hz), 6.72–6.87 m (4H, $H^{8,10,14,16}{}_{Ar}$), 7.03–7.07 m (1H, $H^{11}{}_{Ar}$), 7.15–7.18 m (1H, $H^{17}{}_{Ar}$), 7.25 t (2H, $H^{9,15}{}_{Ar}$, ^3J = 7.8 Hz), 7.50 br. s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 40.01 (C^4), 57.67 (C^5), 115.63 (C^8), 115.91 (C^{14}), 116.41 (C^{10}), 117.45 (C^{16}), 119.50 ($C^{6,12}$), 127.38 (C^{17}), 128.06 (C^{15}), 128.54 (C^9), 130.00 (C^{11}), 153.46 (C^3), 155.33 (C^{13}), 157.28 (C^7).

4-[5-(4-Methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-3-yl]benz-1,3-diol (9). Yield 37%, mp 149–150°C. ^{1}H NMR spectrum, δ, ppm: 2.84 d. d (1H, H^{4ax}, ^2J = 11.0, ^3J = 11.0 Hz), 3.43 d. d (1H, H^{4eq}, ^2J = 11.0, ^3J = 5.3 Hz), 3.70 s (3H, H^{21}), 4.68 t (1H, H^5, ^3J = 10.5 Hz), 6.27 m (2H, $H^{8,10}$), 6.87 d (2H, $H^{14,16}$, ^3J = 8.7 Hz), 7.05 d (1H, H^{11}, ^3J = 8.7 Hz), 7.27 d (2H, $H^{13,17}$, ^3J = 8.7 Hz), 11.22 br. s (1H, NH). ^{13}C NMR spectrum, δ_C, ppm: 41.59 (C^4), 55.62 (CH_3), 61.86 (C^5), 102.92 (C^8), 107.50 (C^{10}), 109.44 (C^6), 114.35 ($C^{14,16}$), 128.39 ($C^{13,17}$), 129.40 (C^{11}), 134.76 (C^{12}), 153.87 (C^3), 159.09 (C^9), 159.74 (C^{15}), 162.10 (C^7).

2-[5-(4-Hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]phenol (10). Yield 89%, mp 110–111°C. ^{1}H NMR spectrum, δ, ppm: 2.89 d. d (1H, H^{4ax}, ^2J = 16.2, ^3J =

10.8 Hz), 3.47 d. d (1H, H^{4eq}, ^2J = 16.2, ^3J = 11.0 Hz), 4.69 t (1H, H^5, ^3J = 9.8 Hz), 6.70 d (2H, $H^{14,16}$, ^3J = 7.3 Hz), 6.81–6.87 m (2H, $H^{8,10}$), 7.13–7.19 m (3H, $H^{9,13,17}$), 7.24 d (2H, H^{11}, ^3J = 7.3 Hz), 7.68 br. s (1H, NH), 9.35 s (1H, OH^{19}), 11.16 s (1H, OH^{18}). ^{13}C NMR spectrum, δ_C, ppm: 41.24 (C^4), 62.36 (C^5), 115.68 ($C^{14,16}$), 116.23 (C^8), 117.35 (C^6), 119.65 (C^{10}), 128.25 (C^{11}), 128.37 ($C^{13,17}$), 130.17 (C^9), 132.79 (C^{12}), 153.06 (C^3), 157.22 (C^{15}), 157.26 (C^7).

2-Ethoxy-4-[3-(2-hydroxyphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl]phenol (11). Yield 93%, mp 89–90°C. ^1H NMR spectrum, δ, ppm: 1.27 t (3H, H^{21}, ^3J = 7.3 Hz), 2.92 d. d (1H, H^{4ax}, ^2J = 16.7, ^3J = 11.0 Hz), 3.49 d. d (1H, H^{4eq}, ^2J = 16.7, ^3J = 11.0 Hz), 3.96 q (2H, H^{20}, ^3J = 6.7 Hz), 4.69 t (1H, H^5, ^3J = 11.0 Hz), 6.69–6.74 m (2H, $H^{10,14}$), 6.82–6.92 m (3H, $H^{8,13,17}$), 7.16 d (1H, H^{11}, ^3J = 7.3 Hz), 7.25 t (1H, H^9, ^3J = 7.3 Hz), 7.71 s (1H, NH), 8.87 br. s (1H, OH^{19}), 11.16 s (1H, OH^{18}). ^{13}C NMR spectrum, δ_C, ppm: 15.32 (C^{21}), 41.31 (C^4), 62.65 (C^5), 64.37 (C^{20}), 112.75 (C^{17}), 115.88 (C^{10}), 116.23 (C^8), 117.37 (C^6), 119.65 (C^{13}), 119.69 (C^{14}), 128.26 (C^9), 130.18 (C^{11}), 133.29 (C^{12}), 146.68 (C^{15}), 147.17 (C^{16}), 153.16 (C^3), 157.26 (C^7).

Flavonones 12–15 (*general procedure*). A mixture of 0.001 mol of substituted chalcone and catalytic amount of triethylamine in 15 mL of 95% ethanol was refluxed for 8 h. The precipitate was filtered off and dried at room temperature.

2-(2-Hydroxyphenyl)flavon-4-one (12). Yield 94%, mp 147–148°C. ^1H NMR spectrum, δ, ppm: 2.76 d. d (1H, H^{3ax}, ^2J = 16.9, ^3J = 2.7 Hz), 3.14 d. d (1H, H^{3eq}, ^2J = 16.9, ^3J = 13.3 Hz), 5.75 d. d (1H, H^2, ^3J = 13.3, 2.8 Hz), 6.77–6.83 m (3H, $H^{13,14,15}$), 7.05 d (1H, H^{16}, ^3J = 7.8 Hz), 6.86 d (1H, H^7, ^3J = 8.2 Hz), 7.13 t (1H, H^{10}, ^3J = 8.2 Hz), 7.49 t (1H, H^8, ^3J = 7.8 Hz), 7.54 t (1H, H^9, ^3J = 6.9 Hz), 8.09 s (1H, OH). ^{13}C NMR spectrum, δ_C, ppm: 43.02 (C^3), 74.85 (C^2), 116.31(C^{13}), 118.27 (C^5), 118.71 (C^7), 119.78 (C^{15}), 121.64 (C^8), 122.07 (C^9), 125.58 (C^{16}), 126.89 (C^{10}), 127.34 (C^{14}), 130.04 (C^{11}), 136.79 (C^{12}), 154.84 (C^6), 162.03 (C^4).

7-Hydroxy-2-(4-methoxyphenyl)flavon-4-one (13). Yield 76%, mp 146–147°C. ^1H NMR spectrum, δ, ppm: 2.59 d. d (1H, H^{3ax}, ^2J = 16.8, ^3J = 2.8 Hz), 3.08 d. d (1H, H^{3eq}, ^2J = 16.8, ^3J = 16.1 Hz), 3.71 s (3H, H^{20}), 5.45 d. d (1H, H^2, ^3J = 12.8, 2.3 Hz), 6.29 s (1H, H^7), 6.46 d (1H, H^9, ^3J = 8.0 Hz), 6.97 d (2H, $H^{13,15}$, ^3J = 8.2 Hz), 7.39 d (2H, $H^{12,16}$, ^3J = 8.7 Hz), 8.14 d (1H, H^{10}, ^3J = 8.7 Hz), 10.62 br. s (1H, OH^{18}). ^{13}C NMR spectrum, δ_C, ppm: 43.67 (C^3), 55.65 (C^{20}), 79.25 (C^2), 103.09 (C^7), 111.08 (C^9), 114.33 ($C^{13,15}$), 114.94 (C^5), 128.74 ($C^{12,16}$), 131.54 (C^{10}), 133.51 (C^{11}), 159.85 (C^{14}), 165.16 (C^6), 166.34 (C^8), 190.59 (C^4).

2-(4-Hydroxyphenyl)flavon-4-one (14). Yield 95%, mp 184–185°C. ^1H NMR spectrum, δ, ppm: 2.73 d. d (1H, H^{3ax}, ^2J = 16.7, ^3J = 3.2 Hz), 3.18 d. d (1H, H^{3eq}, ^2J = 16.7, ^3J = 12.8 Hz), 5.48 d. d (1H, H^2, ^3J = 12.8, 2.8 Hz), 6.77 d (2H, $H^{13,15}$, ^3J = 8.2 Hz), 7.30 d (2H, $H^{12,16}$, ^3J = 8.3 Hz), 7.00–7.05 m (2H, $H^{7,9}$), 7.52 t (1H, H^8, ^3J = 8.2 Hz), 7.75 d (1H, H^{10}, ^3J = 7.9 Hz), 9.48 br. s (1H, OH^{18}). ^{13}C NMR spectrum, δ_C, ppm: 43.94 (C^3), 79.40 (C^2), 115.82 (C^{13}), 115.92 (C^{15}), 118.76 ($C^{7,9}$), 121.19(C^5), 128.54 (C^{12}), 128.91 (C^{16}), 129.69 (C^{11}), 136.80 (C^8), 158.19 (C^{14}), 161.77 (C^6), 192.40 (C^4).

2-(3-Ethoxy-4-hydroxyphenyl)flavon-4-one (15). Yield 96%, mp 127–128°C. ^1H NMR spectrum, δ, ppm: 1.29 t (3H, H^{20}, ^3J = 6.9 Hz), 2.71 d. d (1H, H^{3ax}, ^2J = 17.0, ^3J = 2.7 Hz), 3.26 d. d (1H, H^{3eq}, ^2J = 17.0, ^3J = 13.3 Hz), 4.00 q (2H, H^{19}, ^3J = 6.9 Hz), 5.47 d. d (1H, H^2, ^3J = 12.8, 2.8 Hz), 6.78 d (1H, H^{16}, ^3J = 8.2 Hz), 6.89 d (1H, H^{12}, ^3J = 8.2 Hz), 7.02–7.06 m (3H, $H^{7,10,15}$), 7.53 t (1H, H^8, ^3J = 8.2 Hz), 7.76 t (1H, H^9, ^3J = 7.8 Hz), 9.00 s (1H, OH). ^{13}C NMR spectrum, δ_C, ppm: 15.29 (C^{20}), 44.03 (C^3), 64.55 (C^{19}), 79.57 (C^2), 113.10 (C^{12}), 115.86 (C^{15}), 118.57 (C^7), 120.20 (C^{16}), 121.15 (C^5), 121.81 (H^9), 126.81 (C^{10}), 130.18 (C^{11}), 136.71 ($C^8{}_a$), 147.20 (C^{14}), 147.77 (C^{13}), 161.76 (C^6), 192.47 (C^4).

Biological tests. Anti-inflammatory effect (*in vitro*) of the investigated compounds was estimated as the ability to inhibit lipopolysaccharide-induced production of anti-inflammatory cytokines interluekin-6 and tumor necrosis factor in monocytic MonoMac-6 cell as well as NF-κB-dependent production of alkali phosphatase in the transfected monocytic THP-1Blue cells. The cells were treated with a tested compound for 30 min, then bacterial lipopolysaccharide extracted from *Escherichia coli* (Sigma-Aldrich, USA) was added to final concentration 0.5 μg/mL. After 24-hours incubation of the cells in CO_2-incubator (37°C), concentrations of the cytokines in the cell supernatants were measured using enzyme immunoassay, then alkali phosphatase production was measured using specific substrate Quanti-Blue™ (Promega, USA). Cytotoxicity level of the investigated compounds was estimated using a CellTiter-Glo™ chemiluminescent set (Promega, USA). Effective concentration causing inhibition of biological response (cytokine and alkali phosphatase production or cytotoxicity) by 50% (IC_{50}) was found

using regression analysis of the dose-dependent curves (at least 5 concentrations).

Molecular modeling and SAR analysis. Structural models of the investigated molecules were created using HyperChem software (Hypercube, Inc., Gainesville, FL, USA). Physical and chemical parameters (S, log P, E_h, V, α) of compounds **1**, **3**–**5**, **8**–**10**, **12**, **14**, and **15** were calculated using QSAR module implemented in HyperChem 7. Linear discriminant analysis and search for regression model (5) were performed using STATISTICA software package (StatSoft, Inc., Tulsa, OK, USA).

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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