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Synthesis of New Combretastatin A-4 Analogues and Study of Their Anti-Inflammatory Activity

M. P. Davydova[a], I. V. Sorokina[b], T. G. Tolstikova[b], V. I. Mamatyuk[b, c], D. S. Fadeev[b], and S. F. Vasilevsky[b, c, 1]

[a] *Voevodsky Institute of Chemical Kinetics and Combustion, Siberian Branch, Russian Academy of Sciences, ul. Institutskaya 3, Novosibirsk, 630090 Russia*

[b] *Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch, Russian Academy of Sciences, pr. Lavrent'eva 9, Novosibirsk, 630090 Russia*

[c] *Novosibirsk State University, ul. Pirogova 2, Novosibirsk, 630090 Russia*

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Abstract—A new approach to the synthesis of natural combretastatin A-4 analogues based on the interaction of α-acetylenic ketones with secondary amines (diethyl amine, pyrrolidine, piperidine, morpholine) was proposed. Previously unknown analogues of combretastatin A-4 containing the β-aminovinylcarbonyl bridges were synthesized. Anti-inflammatory activity of the obtained compounds was studied on models of exudative inflammation caused by histamine or concanavalin A.

Keywords: combretastatin A-4, α-acetylenic ketones, β-vinylamines, anti-inflammatory activity, vasoactive properties

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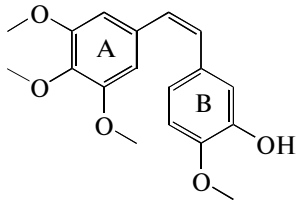
INTRODUCTION

Synthetic modification of plant metabolites is a topical trend in medicinal chemistry. One of the promising compounds exhibiting the inflammatory activity is combretastatin A-4 (CA-4), the derivative of cis-stilbene, which was isolated for the first time 25 years ago from the South African tree *Combretum caffrum* by Pettit and coworkers [1]. Due to its simple molecular structure and the simple synthesis of its analogues, CA-4 has attracted the attention of chemists and pharmacologists in the last two decades. This resulted in the appearance of tens of effective agents related in structure to combretastatin [2–4].



Formula 1. Structure of combretastatin A-4.

According to the literature data [5], combretastatins bind to the colchicine site in β-subunit of α,β-heterodimers of tubulin and inhibit its polymerization in microtubes. Violation of the tubulin polymerization causes spindle cell lesions, inhibition of tumor cells proliferation, and disturbances in the signaling pathways associated with regulation and preservation of cytoskeleton of endothelial cells in tumor vessels. These data promote the synthesis of modified combretastatins in order to search more selective and less toxic efficient vasoactive compounds.

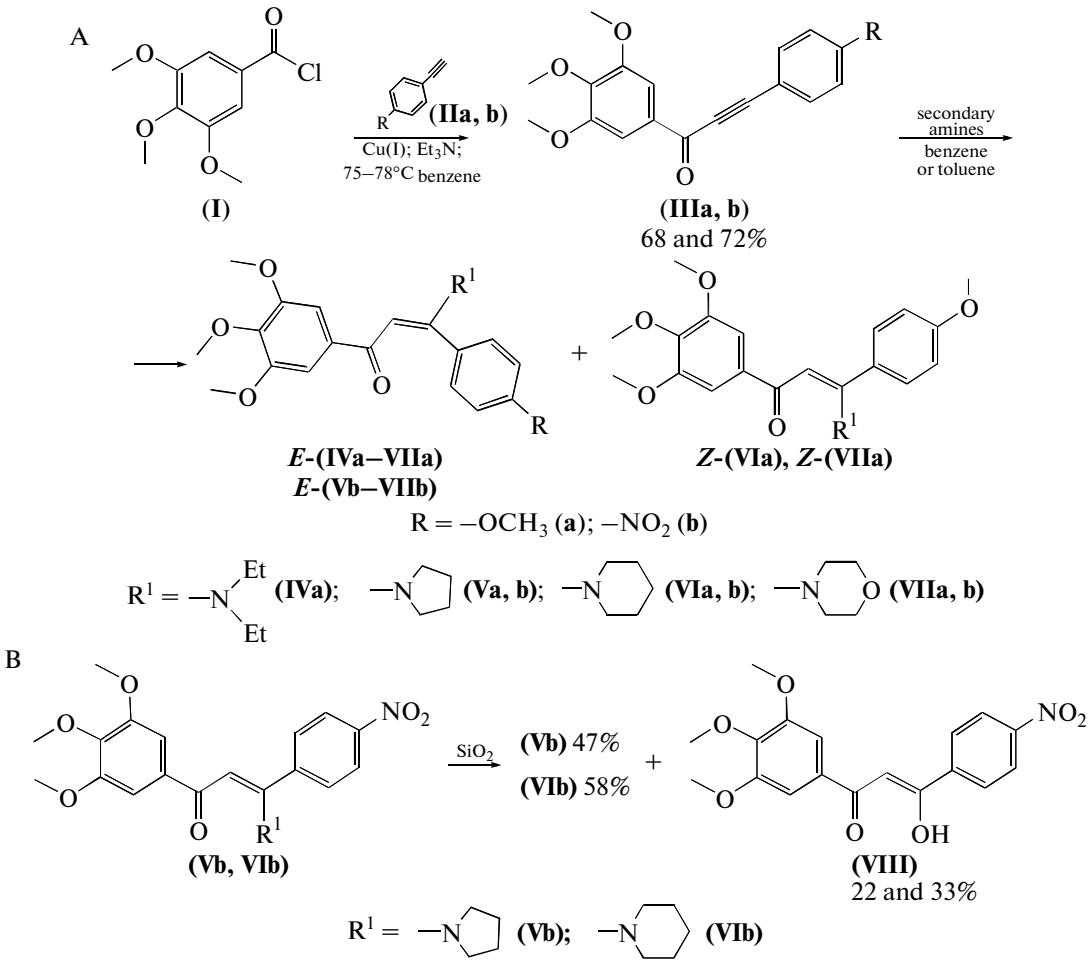
The modification of CA-4 does not usually lead to the transformation of the A ring (3,4,5-trimethoxyphenyl residue), while the B ring changes, with modification of the olefine unit being most important [2, 3]. Most often, the ethylene bridge is replaced by the heterocyclic fragment or the bridge consisting of three carbon atoms. It should be also noted that it is necessary to introduce the functional groups into the linkers between the aromatic rings to enhance hydrophilicity of the molecule because one of the disadvantages of CA-4 and its analogues is a low solubility in water [2, 3, 6].

In our previous work, we described the modification of CA-4 by the formation of the heterocyclic bridge [7]. The goal of the present work is the synthesis of the CA-4 analogues containing a three-carbon spacer with the amine functions and the evaluation of their anti-inflammatory activity. Moreover, we evaluated the vasoactive properties of the synthesized compounds by the increase in exudative edema of mice paws caused by the subplantar administration of histamine or concanavalin A.

RESULTS AND DISCUSSION

The intended analogues of CA-4 were β-aminovinylketones, i.e., 3-amino-3-Ar-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-ones (Ar is the 4-methoxy- or 4-nitrophenyl residue) (Scheme 1, A).

[1] Corresponding author: phone: +7 (383) 333-33-47; fax: +7(383) 330-73-50; e-mail: vasilev@kinetics.nsc.ru.



Scheme 1. Scheme of the synthesis of β-aminovinylketones (**IVa**)–(**VIIa**) and (**Vd**)–(**VIId**) (A) and hydrolysis of vinylamines (**Vb**) and (**VIb**) (B).

A systematic series of β-aminovinylketones for the pharmacological study was obtained starting from acetylenes bearing the electron donor (methoxy) and acceptor (nitro) groups in the arylalkyne fragment of the molecule, with diethylamine, pyrrolidine, morpholine, and piperidine being used as reagents.

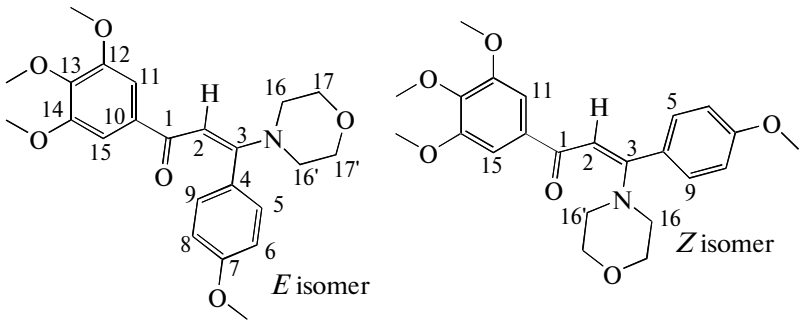
Ketoacetylenes (**IIIa**) and (**IIIb**) were added to the reaction mixture with diethylamine and pyrrolidine in benzene at 55°C and under boiling, respectively, and with morpholine and piperidine, in toluene under boiling. The attachment of amines is completed in 2–10 h resulting in the formation of adducts (**IV**)–(**VII**) in yields of 47–85%. As expected, the reaction of ketoacetylene (**IIIb**) activated with the acceptor nitro group occurred faster in comparison with the other compounds (Scheme 1, A, Table 1).

The purification of nitroderivatives of amines (**Vb**) and (**VIb**) by column silica gel chromatography led to the target aminovinylketones along with a new product, i.e., keto alcohol (**VIII**) (22% and 33%, respectively), which was obviously the result of the hydrolysis of compounds (**Vb**) and (**VIb**) on a sorbent (Scheme 1, B). It should be noted that the hydrolysis of β-aminovinylketones to the corresponding diketones has been described in literature but under more severe conditions, i.e., under the boiling of the alcohol solution of β-aminovinylketones with diluted HCl (1 : 1) for 2 h [9].

The antitumor activity of combretastatins is known to depend on *Z*/*E* isomery [2, 5, 6]. Therefore, we pay particular attention to the configuration of synthesized β-aminovinylketones, which was determined by the NOESY experiments. The *E* isomers demonstrated negative cross-peaks indicating the spatial proximity between proton H2 and protons H11, H15, and aliphatic protons in the α-position of the substituent. The *Z* isomers had cross-peaks corresponding to the interaction of proton H2 with protons H11, H15, H5, and H9. Moreover, the positive cross-peaks (co-directional with the diagonal ones) corresponding to the chemical exchange between the intended nuclei of the *E* and *Z* isomers.

Table 1. Conditions of the reaction of ketoacetylenes (**III**) with secondary amines (scheme) and yields of β-aminovinylketones

Product	Isomer	Reaction time, h	Reaction temperature, °C	Solvent	Yield, %
(IVa)	*E*	8.5	55	Benzene	79
(Va)	*E*	5.5	80	Benzene	65
(Vb)	*E*	2	80	Benzene	58
(VIa)	*E* + *Z*	5	110	Toluene	51
(VIb)	*E*	3	110	Toluene	47
(VIIa)	*E* + *Z*	10	110	Toluene	77
(VIIb)	*E*	5	110	Toluene	85



Formula 2. Structures of *E* and *Z* isomers of compound (**VIIa**).

It should be noted that it is impossible to judge the isomer purity of compounds on the basis of PMR spectra. The PMR data do not show the presence or absence of the stereoselectivity of the process because the dynamic equilibrium between *E* and *Z* isomers may be observed in solutions when recording proton spectra. Indeed, in special experiments with compound (**VIIa**), we showed that the equilibrium concentration of the *E* and *Z* isomers depended on the solvent and temperature. The ratio of the isomers in chloroform and DMSO was 7 : 3 and 81 : 19, respectively, at room temperature. We also observed in DMSO the line broadening of signals in the proton spectra when heating the reaction mixture in the temperature range from 80°C to 130°C ($k = 9.3\ s^{-1}$ at 91°C).

As to the other compounds, their ^{1}H NMR spectra were recorded under the same conditions (chloroform, room temperature), and we observed only the presence of the *E* isomer. The exclusion was compound (**VIa**), the spectrum of which contained the mixture of the *E* and *Z* isomers (7 : 3) (Table 1).

Thus, we have proposed the approach to the synthesis of the analogues of native combretastatin A-4 based on the interaction of α-acetylenic ketones with secondary amines (diethylamine, pyrrolidine, piperidine, morpholine), which leads to new derivatives of CA-4 containing the β-aminovinylcarbonyl bridges.

The side keto alcohol was found to be formed as the result of the silica gel-induced hydrolysis of β-aminovinylketones bearing the nitro group.

Anti-Inflammatory Properties of Combretastatin Analogues

According to the literature data, the vasoactive effects of comretastatines are due, mostly, to the polymerization of tubulin and functional inhibition of the VE-cadgerin/β-catenin complex, which causes the change in the form of endothelial cells, disturbance of their intercellular adhesion and the increase in the permeability of the vascular wall [2]. These features of the action of combretastatins can modify the course of inflammatory processes; in particular, they can enhance the plasma exudation and blood cell migration to the surrounding tissue. Thus, the increase of inflammatory edema in response to combretastatin against the background of other phlogogens may indicate their vasotropic effect. The goal of the present work was to evaluate the influence of the synthesized combretastatin analogues on inflammation induced by histamine and concanavalin A.

It was shown on a model of histamine inflammation (Table 2) that compounds containing the piperidine and morpholine fragments ((**VIa**) and (**VIIb**),

Table 2. Influence of combretastatin A-4 analogues on the amount of inflammatory mice paw edema induced by subplantar injection of histamine

Group	Average edema index	Amount of edema, %	Anti-inflammatory effect, %
Control	$26.4 \pm 1.6^{\#}$	100	0
(VIa)	$25.0 \pm 1.8^{\#}$	94.7	5.3
(VIIb)	$23.8 \pm 1.3^{\#}$	90.1	9.9
(VIIa)	18.7 ± 1.2*	70.8	29.2
(Vb)	18.2 ± 1.4*	68.9	31.1
(VIb)	16.8 ± 1.0**	63.6	36.4
Indometacin	19.0 ± 0.9**	72.0	28.0

*$P < 0.05$ and **$P < 0.01$, respectively, relative to the control group; $^{\#}P < 0.01$ relative to the group with the intraperitoneal administration of indomethacin (20 mg/kg).

Table 3. Influence of combretastatin A-4 analogues on the amount of inflammatory edema of mice paw induced by subplantar injection of concanavalin A

Group	Average edema index	Amount of edema, %	Anti-inflammatory effect, %
Control	$15.1 \pm 1.5^{\#}$	100	0
(VIa)	$19.2 \pm 1.2^{\#\#}$*	127.1	–27.1
(VIIb)	$18.9 \pm 1.2^{\#\#}$**	125.2	–25.2
(VIIa)	$16.0 \pm 1.2^{\#\#}$	106.0	–6.0
(Vb)	$15.9 \pm 0.9^{\#\#}$	105.3	–5.3
(VIb)	$14.5 \pm 1.1^{\#\#}$	96.0	4.0
Diclofenac	10.5 ± 0.6***	69.5	30.5

*$P < 0.05$, **$P = 0.06$, and ***$P < 0.01$, respectively, relative to the control group); $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$, respectively, relative to the group with intraperitoneal administration of diclofenac.

respectively) exhibit no anti-inflammatory activity, while β-aminovinylketones containing pyrrolidine, piperidine, and morpholine residues ((**Vb**), (**VIb**), and (**VIIa**), respectively) reduce the amount of edema on average by a factor of 1.5 as compared with the control (Table 2). This effect has no significant differences with that of indomethacin.

In inflammatory conditions induced by concanavalin A, compounds (**VIa**) and (**VIIb**) bearing piperidine and morpholine fragments, respectively, increase inflammatory edema relative to the control with high probability (Table 3). The other combretastatin derivatives do not have a significant effect on the amount of edema against the background of lectin. The reference preparation, diclofenac, significantly inhibits the inflammatory reaction.

The comparison of the results shows that the synthesized combretastatins have a modifying effect on the inflammatory reactions induced by phlogogens. Compounds containing the piperidine (**VIa**) and morpholine (**VIIb**) fragments are able to enhance the exudative effect of inflammation due to the obvious

increase in the permeability of the vascular wall. On the contrary, β-aminovinylketones bearing the residues of pyrrolidine (**Vb**), piperidine (**VIb**), and morpholine (**VIIa**) either have no significant effect on the amount of edema, or exhibit the anti-inflammatory activity.

It was previously reported [2, 4, 5] that antitubulin properties of combretastatins depended on isomery, i.e., the activity of *cis*-isomers (*Z*-configuration) was significantly higher than that of *trans*-isomers (*E*-configuration). It has been shown [2] that *cis*-orientation of two aromatic rings in CA-4 is an important factor for inhibiting the growth of cancer cells, and its isomerization into the *trans*-form as a result of storage or application quickly leads to a significant reduction of the tubulin polymerization and antitumor activity. Taking into account these results, we collated the NOESY data (Table 1) with the anti-inflammatory activity of the compounds (Tables 2 and 3). We did not find an unambiguous relationship between the spatial conformation of the compounds and their vasotropic properties. Two combretastatins (**VIa**) and (**VIIb**) caused an increased inflammatory edema, and only the former showed the equilibrium concentration of *cis*- and *trans*-isomers in organic solvents, while the latter was in the *trans*-form. However, we should take into account the fact we found that the solvent can influence the isomerization process of combretastatins. This suggests that the ratio of the isomers can change in a water-tween suspension during administration to animals. This assumption requires further experimental evaluation.

Thus, among a number of the newly synthesized combretastatin A-4 analogues, β-aminovinylketones, we identified compounds with the vasoactive properties, which increased the exudative swelling in local inflammation, and found the derivatives with the anti-inflammatory activity.

EXPERIMENTAL

Spectral-analytical studies were performed in The Chemical Service Center for the collective use of the Siberian Branch of the Russian Academy of Sciences.

NMR spectra were recorded on a Bruker AV-400 spectrometer in $CDCl_3$, which was also the internal standard. The assignment of the signals in the NMR spectra was based on 2D-correlations of $^1H-^1H$ (COSY, NOESY) and $^1H-^{13}C$ (HSQC, HMBC). High resolution mass spectra (MC-BP) were recorded on a Thermo Scientific DFS spectrometer (Thermo Electron Co) by direct input (the temperature of the ionization chamber, 220–270°C; voltage, 70 eV). The melting temperatures were determined on a Kofler apparatus. IR spectra (ν, cm^{-1}) were recorded on a Bruker Vector 22 spectrometer in KBr tablets. Chromatography was carried out on silica gel Merck 60 (0.063–0.2 mm); TLC was performed on plates of Silufol UV-254 (Chemapol) and Kieselgel 60 F_{254} (Merck) in a toluene-ethylacetate (5 : 1) system. Compounds on plates were visualized by UV irradiation.

We used morpholine, pyrrolidine, diethylamine, and piperidine (Aldrich). The starting acetylenic ketones (**IIIa**, **IIIb**) were synthesized as described in [7, 8].

(*E*)-3-Diethylamino-3-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (IVa). The mixture of 3-(4-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (**IIIa**) [8] (390 mg, 1.2 mmol) and diethylamine (260 mg, 3.6 mmol) in benzene (12 mL) was kept for 8.5 h at 50–55°C. After completion of the reaction (TLC control), the reaction mixture was cooled, filtered, and evaporated in vacuo. The residue was crystallized from toluene–hexane. The yield of compound (**IVa**) was 380 mg (79%); mp, 127.5–129°C. ^1H NMR: 1.23 (6H, m), 3.29 (4H, m), 3.81 (3H, s, OCH_3), 3.84 (9H, s, $3OCH_3$), 5.86 (1H, s), 6.93 (2H, d, *J* 8.6, ArH), 7.09 (1H, s, ArH), 7.15 (2H, d, *J* 8.6, ArH). Found, %: C 69.18; H 7.37; N 2.95. $C_{23}H_{29}NO_5$. Calculated, %: C 69.15; H 7.32; N 3.51. IR spectrum: 1624 (C=O).

(*E*)-3-(4-Methoxyphenyl)-3-(pyrrolidin-1-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (Va). The mixture of ketone (**IIIa**) (326 mg, 1 mmol) and pyrrolidine (142 mg, 2 mmol) in benzene (10 mL) was boiled for 5.5 h. The solvent was evaporated in vacuo, and the residue was crystallized from benzene. The yield of compound (**Va**) was 260 mg (65%); mp, 102.5–103.5°C. ^1H NMR: 1.85–2.15 (4H, m), 3.13–3.46 (4H, m), 3.82 (3H, s, OCH_3), 3.86 (9H, s, $3OCH_3$), 5.73 (1H, s), 6.94 (2H, d, *J* 8.6, ArH), 7.14 (1H, s, ArH), 7.19 (2H, d, *J* 8.6, ArH). MC-BP: found, *m/z* 397.1886 $[M]^+$. $C_{23}H_{27}NO_5$; calculated, M = 397.1884. IR spectrum: 1612 (C=O).

(*E,Z*)-3-(4-Methoxyphenyl)-3-(piperidin-1-yl)-1-(3,4,5-trimethoxyphenyl)prop-2en-1-one (VIa). The mixture of ketone (**IIIa**) (280 mg, 0.86 mmol) and piperidine (146 mg, 1.71 mmol) in toluene (10 mL) was boiled for 5 h and evaporated in vacuo. Compound (**VIa**) was obtained in a yield of 51% (180 mg) as a mixture of two isomers (*E*/*Z*, 7 : 3) with mp of 85–87°C.

***E* isomer.** ^1H NMR: 1.62 (4H, m, H17, H19), 1.67 (2H, m, H18), 3.27 (4H, m, H16, H20), 3.80 (3H, s, 7-OCH_3), 3.85 (6H, s, 12,14-OCH_3av), 3.87 (3H, s, 13-OCH_3), 5.87 (1H, s, H2), 6.88 (2H, m, H8), 7.10 (2H, s, H11, H15), 7.19 (2H, m, H5, H9).

***Z* isomer.** ^1H NMR: 1.63 (4H, m, H17, H19), 1.66 (2H, m, H18), 3.30 (4H, m, H16, H20), 3.81 (3H, s, 7-OCH_3), 3.84 (6H, s, 12,14-OCH_3av), 3.86 (3H, s, 13-OCH_3), 5.55 (1H, s, H2), 6.90 (2H, m, H6, H8), 7.14 (2H, s, H11, H15), 7.34 (2H, m, H5, H9).

MC-BP: found, *m/z* 411.2040 $[M]^+$. $C_{24}H_{29}NO_5$; calculated, M = 411.2039. IR spectrum: 1608 (C=O).

(*E,Z*)-3-(4-methoxyphenyl)-3-morpholino-1-(3,4,5-trimethoxyphenyl)prop-2en-1-one (VIIa). The mix-

ture of ketone (**IIIa**) (326 mg, 1 mmol) and morpholine (170 mg, 2 mmol) in toluene (10 mL) was boiled for 10 h and evaporated in vacuo. The product was crystallized from benzene. Compound (**VIIa**) was obtained in a yield of 70% (290 mg) as a mixture of two isomers (*E*/*Z*, 7 : 3), mp 153–155°C.

***E* isomer.** ^{1}H NMR: 3.23 (4H, m, H16, H16'), 3.73 (4H, m, H17, H17'), 3.80 (3H, s, 7-OCH$_3$), 3.85 (6H, s, 12,14-OCH$_3$ av), 3.87 (3H, s, 13-OCH$_3$), 5.87 (1H, s, H2), 6.89 (2H, m, J_1 0.3, J_2 2.2, J_3 8.5, H6, H8), 7.09 (2H, s, H11, H15), 7.19 (2H, m, J_1 0.3, J_2 2.8, J_3 8.5, H5, H9). ^{13}C NMR ($CDCl_3$): 48.67 (C16, C16'), 55.41 (7-O$\underline{C}$H$_3$), 56.47 (12,14-O$\underline{C}$H$_3$), 61.08 (13-O$\underline{C}$H$_3$), 66.83 (C17, C17'), 97.29 (C2), 105.54 (C11, C15), 114.34 (C6, C8), 128.10 (C4), 130.47 (C5, C9), 136.88 (C10), 141.07 (C13), 152.92 (C12, C14), 160.63 (C7), 165.00 (C3), 188.56 (C1).

***Z* isomer.** ^{1}H NMR: 3.39 (4H, m, H16, H16'), 3.83 (4H, m, H17, H17'), 3.84 (3H, s, 7-OCH$_3$), 3.84 (6H, s, 12,14-OCH$_3$ av), 3.86 (3H, s, 13-OCH$_3$), 5.61 (1H, s, H2), 6.92 (2H, m, J_1 0.3, J_2 2.1, J_3 8.5, H6, H8), 7.14 (2H, s, H11, H15), 7.42 (2H, m, J_1 0.3, J_2 2.6, J_3 8.5, H5, H9). ^{13}C NMR: 52.44 (C16, C16'), 55.68 (7-O$\underline{C}$H$_3$), 56.39 (12,14-O$\underline{C}$H$_3$), 61.11 (13-O$\underline{C}$H$_3$), 67.62 (C17, C17'), 97.68 (C2), 105.25 (C11, C15), 114.14 (C6, C8), 130.68 (C4), 131.59 (C5, C9), 136.90 (C10), 140.81 (C13), 153.06 (C12, C14), 161.72 (C7), 164.54 (C3), 184.74 (C1).

MC-BP: found, *m/z* 413.1833 $[M]^+$. $C_{23}H_{27}NO_6$; calculated, M = 413.1830. IR spectrum: 1627 (C=O).

Interaction of 3-(4-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-in-1-on (IIIb) with pyrrolidine. The mixture of ketone (**IIIb**) [7] (170 mg, 0.5 mmol) and pyrrolidine (70 mg, 1 mmol) in benzene (10 mL) was boiled for 2 h. After the completion of the reaction (TLC control), the solvent was removed in vacuo.

3-Hydroxy-3-(4-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (VIII) was isolated by column chromatography (toluene, toluene-ethylacetate, ethylacetate) in a yield of 22% (40 mg), mp 203–205°C (benzene-hexane). ^{1}H NMR: 3.93 (3H, s, OCH$_3$), 3.95 (6H, s, 2OCH$_3$), 6.78 (1H, s), 7.23 (2H, s), 8.11 (2H, d, *J* 8.8, ArH), 8.32 (2H, d, *J* 8.8, ArH), 16.74 (OH, s, 1H). ^{13}C NMR: 56.33, 60.95, 93.97, 104.89, 123.77, 127.85, 130.41, 140.70, 142.69, 149.71, 153.22, 180.19, 187.98. MC-BP: found, *m/z* 359.1000 $[M]^+$. $C_{18}H_{17}NO_7$; calculated, M = 359.0999. IR spectrum, ν,cm^{-1}: 1591 (C=O); 3425 (OH).

(*E*)-3-(4-Nitrophenyl)-3-(pyrrolidine-1-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (Vb) was synthesized with a yield of 58% (120 mg), mp 185–186.5°C (benzene). ^{1}H NMR: 1.88 (2H, br.m, H17, H17'), 2.08 (2H, br.m, H17, H17'), 3.03 (2H, br.m, H16, H16'), 3.49 (2H, br.m, H16, H16'), 3.84 (9H, s, 12,13,14-OCH$_3$ av), 5.80 (1H, s, H2), 7.09 (2H, s, H11, H15), 7.44 (2H, m, J_1 0.4, J_2 1.8, J_3 8.4, H5, H9), 8.28 (2H, m, J_1 0.4, J_2 2.5, J_3 8.4, H6, H8). ^{13}C NMR: 25.17 (C17, C17'), 25.66 (C17, C17'), 49.13 (C16, C16'), 50.21 (C16, C16'), 56.46 (12,14-O$\underline{C}$H$_3$), 61.09 (13-O$\underline{C}$H$_3$), 93.29 (C2), 105.26 (C11, C15), 124.30 (C6, C8), 128.52 (C5, C9), 136.75 (C10), 141.06 (C13), 145.53 (C4), 147.72 (C7), 152.94 (C12, C14), 159.73 (C3), 186.01 (C1). MC-BP: found, *m/z* 412.1626 $[M]^+$. $C_{22}H_{24}N_2O_6$; calculated, M = 412.1629. IR spectrum: 1622 (C=O).

Interaction of 3-(4-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-in-1-one (Ib) with piperidine. The mixture of ketone (**IIIb**) (170 mg, 0.5 mmol) and piperidine (85 mg, 1 mmol) in toluene (5 mL) was boiled for 3 h (TLC control). 3-Hydroxy-3-(4-3-(4-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-in-1-one (**VIII**) was isolated as in the previous procedure.

3-Hydroxy-3-(4-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (VIII) was prepared in a yield of 33% (60 mg), mp 202–204°C (benzene–hexane). For spectral characteristics see above.

(*E*)-3-(4-Nitrophenyl)-3-(piperidine-1-yl)-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (VIb) was prepared in a yield of 47% (100 mg) with mp 137–139°C (benzene). ^{1}H NMR: 1.63 (4H, m, H17, H19), 1.69 (2H, m, H18), 3.23 (4H, m, H16, H20), 3.85 (9H, s, 12,13,14-OCH$_3$), 6.00 (1H, s, H2), 7.07 (2H, m, J_1 0.3, J_2 1.9, J_3 8.5, H5, H9), 7.43 (2H, s, H11, H15), 8.27 (2H, m, J_1 0.3, J_2 2.4, J_3 8.5, H6, H8). ^{13}C NMR: 24.37 (C18), 25.94 (C17, C19), 49.52 (C16, C20), 56.52 (12,14-O$\underline{C}$H$_3$), 61.10 (13-O$\underline{C}$H$_3$), 95.46 (C2), 105.35 (C11, C15), 124.19 (C6, C8), 129.46 (C5, C9), 136.56 (C10), 141.30 (C13), 144.72 (C4), 148.09 (C7), 153.03 (C12, C14), 162.29 (C3), 187.34 (C1). Found, %: C 64.62; H 5.90; N 6.46. $C_{23}H_{26}N_2O_6$. Calculated, %: C 64.78; H 6.15; N 6.57. IR spectrum: 1625 (C=O).

(*E*)-3-(4-Nitrophenyl)-3-morpholino-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (VIIb). The mixture of ketone (**IIIb**) (341 mg, 1 mmol) and morpholine (174 mg, 2 mmol) in toluene (10 mL) was boiled for 5 h. After the completion of the reaction, the solvent was removed in vacuum, and the product (**VIIb**) was crystallized from benzene with a yield of 85% (365 mg), mp 195–197°C. ^{1}H NMR: 3.19 (4H, m, H16, H16'), 3.74 (4H, m, H17, H17'), 3.85 (9H, s, 12,13,14-OCH$_3$), 6.03 (1H, s, H2), 7.07 (2H, s, H11, H15), 7.44 (2H, m, J_1 0.4, J_2 1.8, J_3 8.4, H5, H9), 8.26 (2H, m, J_1 0.4, J_2 2.5, J_3 8.4, H6, H8). ^{13}C NMR: 48.36 (C16, C16'), 56.51 (12,14-O$\underline{C}$H$_3$), 61.11 (13-O$\underline{C}$H$_3$), 66.51 (C17, C17'), 97.34 (C2), 105.50 (C11, C15), 124.24 (C6, C8), 129.72 (C5, C9), 135.87 (C10), 141.68 (C13), 143.70 (C4), 148.21 (C7), 153.08 (C12, C14), 162.10 (C3), 187.67 (C1). MC-BP: found, *m/z* 428.1578 $[M]^+$. $C_{22}H_{24}N_2O_7$; calculated, M = 428.1574. IR spectrum: 1639 (C=O).

Histamine inflammation. We used in the experiment 72 outbred mice, i.e., males (nine groups of eight animals) with mass of 25–30 g. The studied compounds were administrated intraperitoneally as a suspension in distilled water with addition of emulsifier

Tween-80 at an effective dose of 20 mg/kg. A separate group of mice and the control group obtained similarly injected reference drug indomethacin (Fluka) and water, respectively, at the same dose. One hour after the administration, 0.01% water solution of histamine (0.05 mL/mouse) was administered by subplantar injection in the hind paw of all mice. Five hours after administration of phlogogen, mice were sacrificed by cervical spine dislocation, both hind paws were cut off, and the mass of each was determined. The anti-inflammatory effect was evaluated by the inflammation index, which was the ratio (%) of the mass difference between the inflamed and intact paws to the mass of intact paw. The results were statistically processed using the STATISTIKA 8 software package.

Concanavalin inflammation. The inflammatory edema was induced in 72 outbred mice (25–30 g) by the injection of concanavalin A solution (0.02 mL, 5 mg/mL) to the aponeurosis of the hind paw. One hour before the administration of lectine, the mice of the experimental groups received intraperitoneally administered the agents in the form of water-tween suspension at a dose of 20 mg/kg. The reference group received diclofenac at the same dose. One hour after the administration of concanavalin A, mice were sacrificed by cervical dislocation, and the paw edema index was determined as described above.

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