Synthesis and antimitotic activity of alkoxy-substituted 1-aryl-3-(arylamino)alkenones

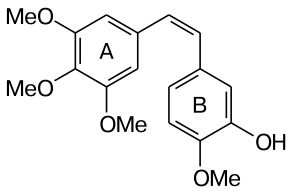
A. V. Samet,[a] V. Yu. Zhuzhin,[b] M. N. Semenova,[c] and V. V. Semenov[a]*

[a]N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation. Fax: +7 (495) 135 5328. E-mail: sametav@server.ioc.ac.ru
[b]Higher Chemical College, Russian Academy of Sciences, Build. 1, 9 Miusskaya pl., 125047 Moscow, Russian Federation
[c]N. K. Koltsov Institute of Developmental Biology, Russian Academy of Sciences, 26 ul. Vavilova, 119334 Moscow, Russian Federation

Alkoxy-substituted 1-aryl-3-(arylamino)prop-2-en-1-ones and 1-aryl-3-(arylamino)but-2-en-1-ones were synthesized by the addition of anilines to 1,3-dioxo derivatives or aryl ethynyl ketones. The *cis-trans* isomerism of these compounds was studied. Biological tests on a sea urchin embryo model showed that 1-aryl-3-(arylamino)prop-2-en-1-ones exhibit an antimitotic effect both *via* microtubule destabilization and through the action on other cellular targets. The antiproliferative activity of these compounds increases with an increase in the number of alkoxy substituents in 1-aryl ring, whereas the presence of one methoxy group in the *para* position proved to be optimal for the arylamino moiety.

Key words: 1-aryl-3-(arylamino)prop-2-en-1-ones, 1-aryl-3-(arylamino)but-2-en-1-ones, *cis-trans* isomerism, tubulin, antimitotic activity, sea urchin embryos.

The natural antimitotic agent combretastatin A-4 (CA4) is a powerful tubulin polymerization inhibitor that binds at the colchicine site.[1,2] The combretastatin derivative phosphorylated at the hydroxy group is currently under clinical trials as an antitumor drug.[3,4] The simplicity of the CA4 structure makes it possible to synthesize numerous analogs of this compound, which do not undergo *cis-trans* isomerism responsible for the loss of antimitotic activity.[5] In particular, earlier we have shown that such compounds can be synthesized from allylpolyoxybenzenes derived from plant raw materials (parsley and dill essential oils).[6—8]



CA4

Numerous studies[9—11] demonstrated that the presence of electron-donating substituents OR or NR_2 and the *cis* arrangement of benzene rings with respect to the bridge X that links these rings are key structural factors responsible for the antimitotic activity of CA4 derivatives. Not only the —C=C— bond that is present in CA4 but also two-atom groups, such as $—(CH_2)_2—$, $—CH_2NH—$, and —CONH—,[12—14] carbocycles or heterocycles (usually five-membered),[15—18] one- and three-atom moieties, such as —C(=O)— and —C=C—C(=O)—,[8,19—21] and some other groups (structure **I**)[9—11] can serve as the bridge X. Four-atom bridges in combretastatin analogs are less well-studied. Notably, one of isomers of the CA4 analog containing the butadiene moiety —C=C—C=C— in an *E,Z* conformation proved to be a very active tubulin polymerization inhibitor.[22]

Recently, the antitumor properties of CA4 analogs containing another four-atom group, *viz.*, C(=O)—CH=CH—NH, (structure **II**) have been investigated.[23] Unexpectedly, it was found that the resulting (*Z*)-1-aryl-3-arylaminoprop-2-en-1-ones **II** have the microtubule-stabilizing rather than destabilizing effect, which is similar to that exhibited by taxol, and is followed by caspase-dependent apoptosis. In the cited study,[23] the presence of exclusively the *Z* isomer was proved by ^{1}H NMR spectroscopy in $CDCl_3$; however, solutions for biological assays were prepared in DMSO, which could lead to the isomerization typical of CA4 analogs.

The aim of the present work was to synthesize 1-aryl-3-(arylamino)prop-2-en-1-ones **1** and 1-aryl-3-(arylamino)but-2-en-1-ones **2**, in which the benzene rings are linked by the four-atom —C(O)—CR=CH—NH— fragment in order to investigate their stereoisomer-

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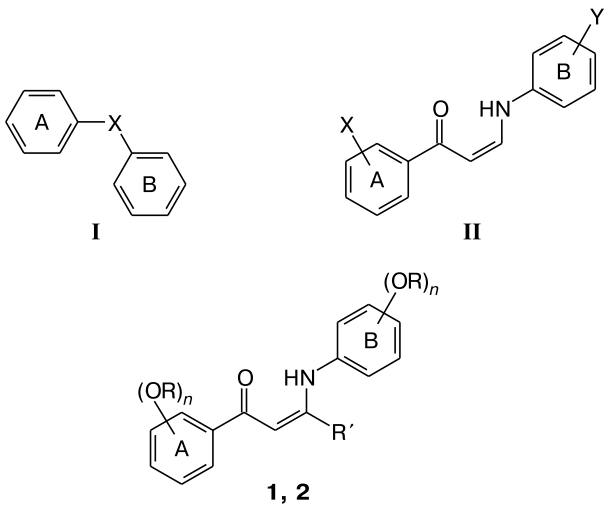


I: X = CH_2, CH_2NH, CO, CONH, COCH=CH, CH=CH—CH=CH, Het, *etc*.

R′ = H (**1**), Me (**2**)

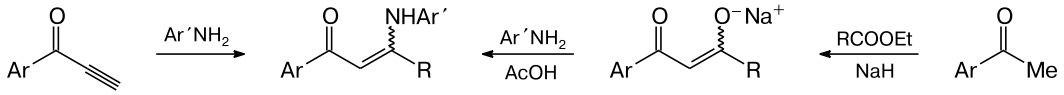
ism and biological action as tubulin polymerization modulators.

The target compounds were synthesized by the Claisen condensation of the corresponding acetophenones **3** with ethyl formate (ethyl acetate) to form benzoylacetaldehydes (benzoylacetones) followed by the reaction of the latter compounds with anilines. In some cases, related compounds were synthesized by the addition of anilines to aryl ethynyl ketones **4** (Scheme 1).

The ^{1}H NMR spectra recorded in DMSO-d_6 (both 1 and 24 h after the dissolution) show that compounds **1** are mixtures of *cis* and *trans* isomers (as a rule, with the former predominating, except for compounds **1e**,**f** containing substituents in the *ortho* position with respect to the bridging C(O)—C=C—NH group), as evidenced by the spin-spin coupling constants (12.4—13.0 Hz for the *trans* isomers and 7.7—7.9 Hz for the *cis* isomers). The chemical shift of the NH proton is *ca*. 12 ppm for the *cis* isomer and *ca*. 10 ppm for the *trans* isomer. Meanwhile, only the *cis* isomer is observed in $CDCl_3$. This is apparently due to the stabilization of the *cis* isomer *via* an intramolecular N-H...O=C hydrogen bond[24—27] (this is confirmed also by a downfield shift of the signal of the NH proton in the spectrum of the *cis* isomer), which is partially cleaved in the polar solvent DMSO-d_6. Compounds **2** exist exclusively as *cis* isomers.[24] This follows from the chemical shifts for the NH protons in the ^{1}H NMR spectra of **2** (in DMSO-d_6), which are similar to those for the NH protons in the *cis* isomers (but not in the *trans* isomers) of compounds **1**. It should be noted that the isomeric composition of products **1**, which were prepared by the addition of anilines to ethynyl ketones, may differ from that of the same products prepared by the condensation of anilines with benzoylacetaldehydes. For instance, according to the ^{1}H NMR spectroscopic data (in DMSO-d_6, 1 h after the dissolution), **1b** generated from ethynyl ketone **4** (Ar = 4-$MeOC_6H_4$) exists as a single isomer (*cis*). After overnight storage, this isomer is transformed into a mixture of isomers of the same composition as for **1b**, which was prepared from acetophenone **3a** through the corresponding benzoylacetaldehyde (*cis*/*trans* is 7 : 3, this ratio was observed both 1 and 24 h after the dissolution). Meanwhile, according to the ^{1}H NMR spectroscopic data (in DMSO-d_6), compound **1a** exists as a mixture of isomers of the *cis*/*trans* composition equal to 2 : 1 (5 min, 1 h, and 24 h after the dissolution) regardless of the method used for the synthesis of **1a**.

The biological activity of the compounds was examined on embryos of the sea urchin *Paracentrotus lividus* using the phenotypic method developed in our earlier study.[28] This method allows for the detection of compounds capable of disturbing cell division and provides information on the mechanism of antimitotic activity. The characteristic change in the embryo motility — the spinning on the bottom of a vessel instead of forward swimming near the surface — attests to the ability of compounds to bind to tubulin and destabilize microtubules.[28]

Scheme 1



R = H (**1**), Me (**2**)

1, **2**	Ar	Ar′
a	4-$MeOC_6H_4$	4-$MeOC_6H_4$
b	4-$MeOC_6H_4$	3,4,5-$(MeO)_3C_6H_2$
c	3,4-$(MeO)_2C_6H_3$	4-$MeOC_6H_4$
d	3,4,5-$(MeO)_3C_6H_2$	4-$MeOC_6H_4$
e	2,5-$(MeO)_2$-3,4-$(OCH_2O)C_6H$	4-$MeOC_6H_4$
f	2-Br-3,4-$(MeO)_2C_6H_2$	4-$MeOC_6H_4$

3a—f, **4**	Ar
a	4-$MeOC_6H_4$
b	4-$MeOC_6H_4$
c	3,4-$(MeO)_2C_6H_3$
d	3,4,5-$(MeO)_3C_6H_2$
e	2,5-$(MeO)_2$-3,4-$(OCH_2O)C_6H$
f	2-Br-3,4-$(MeO)_2C_6H_2$
4	4-$MeOC_6H_4$

Table 1. Action of alkoxy-substituted 1-aryl-3-arylaminoalkenones on sea urchin embryos

Compound	C^*/μmol L^{-1}			
	Cleavage alteration	Cleavage arrest	Embryo spinning	Deciliation
1b	2	>4	>4	>4
2b	2	>4	>4	1
1d	0.02	0.2	2	2
2d	4	>4	>4	2
1e	0.05	0.5	>4	0.5
2e	4	>4	>4	0.5
1f	0.2	2	>4	0.5
CA4	0.002	0.01	0.05	>4

*C are the threshold concentrations causing the effect.

All the tested compounds exhibited the ability to suppress cell division, but they differed in the value and character of the effect (Table 1). The activity of compounds of series **1** increased with an increase in the number of methoxy substituents in the ring A to three or four, whereas the presence of one methoxy group in the *para* position proved to be optimal for the ring B. Compounds **1d**, **1e**, and **1f** had a much stronger effect on the development of sea urchin embryos compared to compound **1b** and caused not only the cleavage alteration but also the complete cleavage arrest. In this case, sea urchin eggs acquired tuberculate shape characteristic of microtubule-destabilizing agents. The spinning of sea urchin embryos, which confirms the microtubule-destabilizing ability, was revealed for the most active compound **1d**. However, we failed to observe embryo spinning in the case of compounds **1e** and **1f**, because the treatment with these compounds after hatching caused embryo immobilization due to deciliation. Compounds of series **2** exhibiting much lower antiproliferative activity have a pronounced ability to cause deciliation. It is known that antimitotic agents — tubulin polymerization inhibitors (microtubule-destabilizing agents) — do not suppress ciliary beating and cannot cause the detachment of cilia from the cell surface.[28—30] Therefore, compounds **1d**, **1e**, and **1f** not only exhibit microtubule-destabilizing activity but also act on other cellular targets.

It should be noted that our *in vivo* studies on sea urchin embryos did not confirm the ability of compounds **1** to act as promoters of tubulin polymerization and microtubule-stabilizing agents, which has been found earlier in *in vitro* experiments.[23]

It can be suggested that the configurational instability of molecules **1** in solutions (*cis*-*trans* isomerization) influences their biological activity, as it occurs in natural CA4.[10]

Experimental

The ^{1}H NMR spectra of compounds **1** and **2** were recorded in DMSO-d_6 and $CDCl_3$ on Bruker AM 300 and Bruker DRX 500 instruments operating at 300.13 and 500.13 MHz, respectively. The spectroscopic data are summarized in Table 2. The yields of the products and the isomer ratios of compounds **1**

Table 2. ^{1}H NMR spectra of compounds **1a—f** and **2a—e**

Compound	^{1}H NMR (δ, J/Hz)			
	C(2)H	C(3)H	NH	Other
1a[a]	6.02 (d, J = 7.8, *cis*); 6.36 (d, J = 12.4, *trans*)	7.76 (dd, J = 12.4, J = 7.8, *cis*); 7.99 (br.s, *trans*)	9.90 (br.s, *trans*); 12.05 (d, J = 12.4, *cis*)	3.73 (s, OMe, *cis*); 3.74 (s, OMe, *trans*); 3.82 (s, OMe, *cis*+*trans*); 6.93 (m, H_{Ar}, *cis*+*trans*); 7.02 (d, H_{Ar}, J = 8.5, *cis*+*trans*); 7.10 (d, H_{Ar}, J = 8.6, *trans*); 7.26 (d, H_{Ar}, J = 8.7, *cis*); 7.84 (d, H_{Ar}, J = 8.5, *trans*); 7.93 (d, H_{Ar}, J = 8.6, *cis*)
1a[b]	5.94 (d, J = 7.8)	7.39 (dd, J = 12.3, J = 7.8)	12.11 (d, J = 12.3)	3.80 (s, 3 H, OMe); 3.87 (s, 3 H, OMe); 6.89 (d, 2 H, H_{Ar}, J = 8.9); 6.94 (d, 2 H, H_{Ar}, J = 8.8); 7.04 (d, 2 H, H_{Ar}, J = 8.9); 7.92 (d, 2 H, H_{Ar}, J = 8.8)
2a[a]	5.97 (s)	—	12.90 (s)	2.08 (s, 3 H, Me); 3.77 (s, 3 H, OMe); 3.82 (s, 3 H, OMe); 6.98 (m, 4 H, H_{Ar}); 7.21 (d, 2 H, H_{Ar}, J = 8.7); 7.89 (d, 2 H, H_{Ar}, J = 8.8)
1b[a]	6.06 (d, J = 7.9, *cis*); 6.41 (d, J = 12.5, *trans*)	7.88 (dd, J = 12.3, J = 7.9, *cis*); 8.08 (br.s, *trans*)	9.93 (br.s, *trans*); 11.98 (d, J = 12.4, *cis*)	3.61 (s, OMe, *trans*); 3.62 (s, OMe, *cis*); 3.80—3.84 (m, OMe, *cis*+*trans*); 6.42 (s, H_{Ar}, *trans*); 6.63 (s, H_{Ar}, *cis*); 7.03 (m, H_{Ar}, *cis*+*trans*); 7.85 (d, H_{Ar}, J = 8.8, *trans*); 7.95 (d, H_{Ar}, J = 8.8, *cis*)
1b[a,c]	6.06 (d, J = 7.9)	7.88 (dd, J = 12.3, J = 7.9)	11.98 (d, J = 12.4)	3.62 (s, 3 H, OMe); 3.82 (s, 6 H, OMe); 3.83 (s, 3 H, OMe); 6.63 (s, 2 H, H_{Ar}); 7.02 (d, 2 H, H_{Ar}, J = 8.8); 7.95 (d, 2 H, H_{Ar}, J = 8.8)

(*to be contined*)

Table 2 (*continued*)

Compound	^{1}H NMR (δ, *J*/Hz)			
	C(2)H	C(3)H	NH	Other
1b[b]	5.98 (d, *J* = 7.9)	7.41 (dd, *J* = 12.2, *J* = 7.9)	12.12 (d, *J* = 12.2)	3.82 (s, 3 H, OMe); 3.87 (s, 9 H, OMe); 6.31 (s, 2 H, H_{Ar}); 6.95 (d, 2 H, H_{Ar}, *J* = 8.8); 7.93 (d, 2 H, H_{Ar}, *J* = 8.8)
2b[a]	6.02 (s)	—	12.98 (s)	2.19 (s, 3 H, Me); 3.66 (s, 3 H, OMe); 3.80 (s, 6 H, OMe); 3.82 (s, 3 H, OMe); 6.58 (s, 2 H, H_{Ar}); 7.00 (d, 2 H, H_{Ar}, *J* = 8.8); 7.91 (d, 2 H, H_{Ar}, *J* = 8.8)
1c[a]	6.06 (d, *J* = 7.8, *cis*); 6.38 (d, *J* = 12.4, *trans*)	7.76 (dd, *J* = 12.4, *J* = 7.8, *cis*); 8.00 (br.s, *trans*)	9.88 (br.s, *trans*); 12.08 (d, *J* = 12.5, *cis*)	3.73 (s, OMe, *trans*); 3.75 (s, OMe, *cis*); 3.81—3.83 (m, OMe, *cis*+*trans*); 6.91—6.96 (m, H_{Ar}, *cis*+*trans*); 7.02—7.05 (m, H_{Ar}, *cis*+*trans*); 7.25 (d, H_{Ar}, *J* = 9.0, *cis*); 7.43 (d, H_{Ar}, *J* = 2.0, *trans*); 7.47 (dd, H_{Ar}, *J* = 8.4, *J* = 2.0, *trans*); 7.50 (d, H_{Ar}, *J* = 2.0, *cis*); 7.58 (dd, H_{Ar}, *J* = 8.4, *J* = 2.0, *cis*)
1c[b]	5.95 (d, *J* = 7.8)	7.40 (dd, *J* = 12.4, *J* = 7.8)	12.12 (d, *J* = 12.4)	3.80 (s, 3 H, OMe); 3.94 (s, 3 H, OMe); 3.96 (s, 3 H, OMe); 6.89 (m, 3 H, H_{Ar}); 7.54 (dd, 1 H, H_{Ar}, *J* = 8.4, *J* = 2.0); 7.56 (d, 1 H, H_{Ar}, *J* = 2.0)
2c[a]	6.01 (s)	—	12.92 (s)	2.11 (s, 3 H, Me); 3.78 (s, 3 H, OMe); 3.82 (s, 6 H, OMe); 6.97 (d, 2 H, H_{Ar}, *J* = 8.8); 7.01 (d, 1 H, H_{Ar}, *J* = 8.4); 7.21 (d, 2 H, H_{Ar}, *J* = 8.8); 7.48 (s, 1 H, H_{Ar}); 7.54 (d, 2 H, H_{Ar}, *J* = 8.4)
1d[a]	6.11 (d, *J* = 7.8, *cis*); 6.37 (d, *J* = 12.4, *trans*)	7.80 (dd, *J* = 12.5, *J* = 7.8, *cis*); 8.03 (br.s, *trans*)	9.99 (br.s, *trans*); 12.15 (d, *J* = 12.6, *cis*)	3.72—3.75 (m, OMe, *cis*+*trans*); 3.85—3.86 (m, OMe, *cis*+*trans*); 6.92 (d, H_{Ar}, *J* = 9.0, *trans*); 6.95 (d, H_{Ar}, *J* = 9.0, *cis*); 7.11 (d, H_{Ar}, *J* = 9.0, *trans*); 7.15 (s, H_{Ar}, *trans*); 7.24 (s, H_{Ar}, *cis*); 7.28 (d, H_{Ar}, *J* = 9.0, *cis*)
1d[b]	5.93 (d, *J* = 7.7)	7.44 (dd, *J* = 12.4, *J* = 7.7)	12.17 (d, *J* = 12.4)	3.81 (s, 3 H, OMe); 3.91 (s, 3 H, OMe); 3.94 (s, 6 H, OMe); 6.90 (d, 2 H, H_{Ar}, *J* = 8.9); 7.05 (d, 2 H, H_{Ar}, *J* = 8.9); 7.20 (s, 2 H, H_{Ar});
2d[a]	6.09 (s)	—	13.0 (s)	2.11 (s, 3 H, Me); 3.72 (s, 3 H, OMe); 3.78 (s, 3 H, OMe); 3.85 (s, 6 H, OMe); 6.98 (d, 2 H, H_{Ar}, *J* = 8.9); 7.21 (s, 2 H, H_{Ar}); 7.23 (d, 2H, H_{Ar}, *J* = 8.9)
1e[a]	5.97 (d, *J* = 7.8, *cis*); 6.16 (d, *J* = 12.5, *trans*)	7.74 (dd, *J* = 12.5, *J* = 7.8, *cis*); 7.85 (br.s, *trans*)	9.88 (br.s, *trans*); 11.96 (d, *J* = 12.5, *cis*)	3.72 (s, OMe, *trans*); 3.74 (s, OMe, *cis*); 3.80 (s, OMe, *trans*); 3.81 (s, OMe, *cis*); 3.82 (s, OMe, *trans*), 3.86 (s, OMe, *cis*); 6.09 (s, SH_2, *trans*); 6.11 (s, SH_2, *cis*); 6.80 (s, H_{Ar}, *trans*); 6.91 (d, H_{Ar}, *J* = 8.9, *trans*); 6.94 (d, H_{Ar}, *J* = 8.9, *cis*); 6.98 (s, H_{Ar}, *cis*); 7.05 (d, H_{Ar}, *J* = 8.9, *trans*); 7.26 (d, *J* = 8.9, H_{Ar}, *cis*)
1e[b]	6.09 (d, *J* = 7.7)	7.36 (dd, *J* = 12.5, *J* = 7.7)	12.06 (d, *J* = 12.5)	3.80 (s, 3 H, OMe); 3.91 (s, 3 H, OMe); 3.94 (s, 3 H, OMe); 6.05 (s, 2 H, SH_2); 6.89 (d, 2 H, H_{Ar}, *J* = 8.9); 7.04 (d, 2 H, H_{Ar}, *J* = 8.9); 7.07 (s, 1 H, H_{Ar})
2e[a]	5.82 (s)	—	12.80 (s)	2.02 (s, 3 H, Me); 3.78 (s, 3 H, OMe); 3.80 (s, 3 H, OMe); 3.85 (s, 3 H, OMe); 6.09 (s, 2 H, SH_2); 6.91 (s, 1 H, H_{Ar}); 6.97 (d, 2 H, H_{Ar}, *J* = 8.9); 7.22 (d, 2 H, H_{Ar}, *J* = 8.9)
1f[a]	5.64 (d, *J* = 7.7, *cis*); 5.78 (d, *J* = 13.0, *trans*)	7.79 (dd, *J* = 12.7, *J* = 7.7, *cis*); 7.60 (br.s, *trans*)	10.0 (br.s, *trans*); 11.81 (d, *J* = 12.7, *cis*)	3.71 (s, OMe, *trans*); 3.75 (s, OMe, *cis*); 3.77 (s, OMe, *trans*); 3.79 (s, OMe, *cis*); 3.81 (s, OMe, *trans*); 3.82 (s, OMe, *cis*); 6.90 (d, H_{Ar}, *J* = 8.7, *trans*); 6.94—7.03 (m, H_{Ar}, *cis*+*trans*); 7.08 (s, H_{Ar}, *cis*); 7.16 (d, H_{Ar}, *J* = 8.9, *trans*); 7.29 (d, H_{Ar}, *J* = 8.9, *cis*)
1f[b]	5.74 (d, *J* = 7.6)	7.36 (dd, *J* = 12.6, *J* = 7.6)	12.06 (d, *J* = 12.6)	3.81 (s, 3 H, OMe); 3.90 (s, 3 H, OMe); 3.91 (s, 3 H, OMe); 6.90 (d, 2 H, H_{Ar}, *J* = 8.9); 7.05 (s, 1 H, H_{Ar}); 7.06 (d, 2 H, H_{Ar}, *J* = 8.9); 7.08 (s, 1 H, H_{Ar})

[a] The spectrum was recorded in DMSO-d_6.
[b] The spectrum was recorded in $CDCl_3$.
[c] The product was obtained from ketone **4**; the spectrum was recorded one hour after the dissolution.

(according to the ^{1}H NMR spectroscopic data in DMSO-d_6) are given below.

Synthesis of 1-aryl-3-arylaminoprop-2-en-1-ones 1a,b from 4-methoxyphenyl ethynyl ketone (4) (general procedure). A solution of compound **4** (1 mol) and the corresponding aniline (1 mol) in ethanol was refluxed for 5 min and then cooled. The bright yellow crystalline precipitate that formed was filtered off.

Synthesis of 1-aryl-3-arylaminoprop-2-en-1-ones 1a—f (general procedure). Sodium hydride (60% suspension in mineral oil, 80 mg, 2 mmol) was added to a solution of the corresponding acetophenone **1** (2 mmol) and HCOOEt (2 mmol) in THF (3 mL). The reaction mixture was stirred for 4 h (in the case of acetophenones **3e**,**f**, for 18 h) and diluted with diethyl ether (3 mL). The resulting suspension was filtered off, and the precipitate was washed with diethyl ether (5 mL) and dried. The Na salt of the corresponding aroylacetaldehyde thus formed was dissolved in EtOH (3 mL). Then the corresponding aniline (2 mmol) and several drops of AcOH were added. The resulting solution was refluxed for 5 min and then cooled. The bright yellow crystalline precipitate that formed was filtered off.

Synthesis of 1-aryl-3-arylaminobut-2-en-1-ones 2a—e (general procedure). Sodium hydride (60% suspension in mineral oil, 80 mg, 2 mmol) was added to a solution of the corresponding acetophenone **1** (2 mmol) and MeCOOEt (2 mmol) in THF (3 mL). The reaction mixture was stirred for 8 h (in the case of acetophenone **3e**, for 24 h) and diluted with diethyl ether (3 mL). The resulting suspension was filtered off, and the precipitate was washed with diethyl ether (5 mL) and dried. The Na salt of the corresponding aroylacetone thus formed was dissolved in EtOH (3 mL). Then the corresponding aniline (2 mmol) and several drops of AcOH were added. The resulting solution was refluxed for 3 h and then cooled. The pale yellow crystalline precipitate that formed was filtered off. Product **2e** was initially obtained as an oil, which was purified by flash chromatography on silica gel (hexane—ethyl acetate, 5 : 1, as the eluent).

1-(4-Methoxyphenyl)-3-(4-methoxyphenylamino)prop-2-en-1-one (1a). Yield 88% (from ethynyl ketone **4**), 69% (from acetophenone **3a**); *cis*/*trans* ratio is 2 : 1. M.p. 185—187 °C (*cf.* lit. data[23]: m.p. 187—188 °C). Found (%): C, 71.83; H, 5.90; N, 5.18. $C_{17}H_{17}NO_3$. Calculated (%): C, 72.07; H, 6.05; N, 4.94.

1-(4-Methoxyphenyl)-3-(4-methoxyphenylamino)but-2-en-1-one (2a). Yield 61%. M.p. 144—145 °C (*cf.* lit. data[31]: m.p. 142—143 °C). Found (%): C, 72.96; H, 6.35; N, 4.88. $C_{18}H_{19}NO_3$. Calculated (%): C, 72.71; H, 6.44; N, 4.71.

1-(4-Methoxyphenyl)-3-(3,4,5-trimethoxyphenylamino)prop-2-en-1-one (1b). Yield 82% (from ethynyl ketone **4**, *cis* isomer), 65% (from acetophenone **3a**, *cis*/*trans* ratio is 7 : 3). M.p. 123—124 °C. Found (%): C, 66.09; H, 5.94; N, 4.29. $C_{19}H_{21}NO_5$. Calculated (%): C, 66.46; H, 6.16; N, 4.08.

1-(4-Methoxyphenyl)-3-(3,4,5-trimethoxyphenylamino)but-2-en-1-one (2b). Yield 55%. M.p. 103—105 °C. Found (%): C, 66.89; H, 6.58; N, 4.13. $C_{20}H_{23}NO_5$. Calculated (%): C, 67.21; H, 6.49; N, 3.92.

1-(3,4-Dimethoxyphenyl)-3-(4-methoxyphenylamino)prop-2-en-1-one (1c). Yield 70%, *cis*/*trans* ratio is 2 : 1. M.p. 155—156 °C. Found (%): C, 69.24; H, 5.95; N, 4.41. $C_{18}H_{19}NO_4$. Calculated (%): C, 68.99; H, 6.11; N, 4.47.

1-(3,4-Dimethoxyphenyl)-3-(4-methoxyphenylamino)but-2-en-1-one (2c). Yield 62%. M.p. 135—136 °C. Found (%): C, 70.00; H, 6.30; N, 4.04. $C_{19}H_{21}NO_4$. Calculated (%): C, 69.71; H, 6.47; N, 4.28.

1-(3,4,5-Trimethoxyphenyl)-3-(4-methoxyphenylamino)prop-2-en-1-one (1d). Yield 72%, *cis*/*trans* ratio is 2 : 1. M.p. 101—103 °C (*cf.* lit. data[23]: m.p. 107—108 °C). Found (%): C, 66.75; H, 5.91; N, 3.85. $C_{19}H_{21}NO_5$. Calculated (%): C, 66.46; H, 6.16; N, 4.08.

1-(3,4,5-Trimethoxyphenyl)-3-(4-methoxyphenylamino)but-2-en-1-one (2d). Yield 59%. M.p. 112 °C. Found (%): C, 66.93; H, 6.57; N, 4.14. $C_{20}H_{23}NO_5$. Calculated (%): C, 67.21; H, 6.49; N, 3.92.

1-[2,5-Dimethoxy-3,4-(methylenedioxy)phenyl]-3-(4-methoxyphenylamino)prop-2-en-1-one (1e). Yield 60%, *cis*/*trans* ratio is 6 : 5. M.p. 118—119 °C. Found (%): C, 64.13; H, 5.17; N, 4.18. $C_{19}H_{19}NO_6$. Calculated (%): C, 63.86; H, 5.36; N, 3.92.

1-[2,5-Dimethoxy-3,4-(methylenedioxy)phenyl]-3-(4-methoxyphenylamino)but-2-en-1-one (2e). Yield 43%. M.p. 93—95 °C. Found (%): C, 64.85; H, 5.52; N, 3.89. $C_{20}H_{21}NO_6$. Calculated (%): C, 64.68; H, 5.70; N, 3.77.

1-(2-Bromo-4,5-dimethoxyphenyl)-3-(4-methoxyphenylamino)prop-2-en-1-one (1f). Yield 58%, *cis*/*trans* ratio is 2 : 3. M.p. 130—132 °C. Found (%): C, 54.91; H, 4.66; Br, 20.08; N, 3.77. $C_{18}H_{18}BrNO_4$. Calculated (%): C, 55.12; H, 4.63; Br, 20.37; N, 3.57.

Investigation of the antiproliferative activity of compounds on the sea urchin embryo model.[28] Experiments were carried out in the Biological laboratory of the N. K. Koltsov Institute of Developmental Biology of the Russian Academy of Sciences in Cyprus. Adult sea urchins *Paracentrotus lividus L.* (*Echinidae, Echinodermata*) were collected in the coastal zone and kept in an aerated seawater aquarium. The spawning was induced by injecting sea urchins with 0.5 *M* KCl (1—2 mL). The eggs were washed with seawater filtered through a nylon filter and fertilized by the addition of several drops of dilute sperm. Embryos (600—2000 per milliliter) were incubated in filtered seawater at room temperature (18—23 °C) to the stage of beginning of active feeding (36—40 h, middle pluteus 2). Throughout the incubation period, the embryo suspension was stirred using a plastic paddle at a rotation speed of 60 rpm driven by an electric motor.

The stock solutions of chemical compounds were prepared in DMSO and 96% ethanol; the maximum studied concentrations of the compounds depended on their solubility. The solubility of the compounds in solvents and seawater was monitored with a microscope.

The compounds were tested in 6-well cell culture plates at the following stages of the development: 1) eggs (10—15 min after the fertilization); 2) free-swimming hatched blastula (9—10 h after the fertilization). An egg suspension (5 mL) was placed in each well, and the corresponding volume of the solution of the compound tested was added to the required final concentration. The maximum concentration of the solvent did not exceed the maximal tolerated concentration (1% for ethanol and 0.05% for DMSO). The tests were done by twofold serial dilutions of the compounds until the effect disappeared. The antiproliferative activity of the compounds was estimated as the lowest (threshold) concentration that alters fertilized egg division. A change in the motility of sea urchin embryos was evaluated after the treatment with the compounds under study at the stage of hatched blastula, when embryos started to swim due to coordinated beating of cilia. The suppression of forward swimming, the settlement to the bottom of the vessel, and the rapid spinning of embryos attest to the ability of the compound under study to destabilize microtubules. The observa-

tions were made with a Biolam LOMO optical microscope (St-Petersburg).

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