Synthesis and biotests of 2-aryl-5-arylmethylidene-substituted 1,3-oxazol-5(4*H*)-ones and *N*-methyl-3,5-dihydro-4*H*-imidazol-4-ones as combretastatin A-4 analogs

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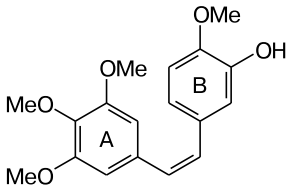
A series of 2-aryl-5-arylmethylidene-1,3-oxazol-5(4*H*)-ones and 2-aryl-5-arylmethylidene-*N*-methyl-3,5-dihydro-4*H*-imidazol-4-ones was synthesized as structural analogs of combretastatin A-4 (a compound possessing antitumor activity). (5*Z*)-5-[(4-Methoxyphenyl)methylidene]-3-methyl-2-(4-methylphenyl)-3,5-dihydro-4*H*-imidazol-4-one was found to exhibit the highest cytotoxicity against cells of human A549 lung carcinoma line ($EC_{50} = 6 \pm 0.8$ μmol L^{-1}).

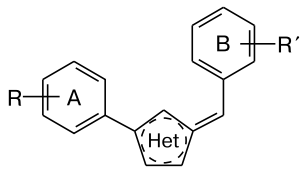
Key words: combretastatin analogs, oxazolones, imidazolones, colchicine domain, tubulin, cytotoxicity.

Development of efficient compounds with simple chemical structure, which makes affordable the corresponding therapy, is an important aspect in the design of antitumor agents. The natural combretastatin A-4 (CA-4) due to its relatively simple structure, very high cytotoxicity, and ability to cause vascular disruption in tumors became a lead compound in the development of numerous series of analogs as potential antitumor agents.[1—5] Combretastatin A-4 binds to a certain site of the dimeric cell protein tubulin at the colchicine domain. That leads to the inhibition of the polymerization process of this protein and prevents the formation of microtubules, which play a key role in the cellular division process.



CA-4

Most analogs of CA-4 were obtained by the replacement of the bridging group in the starting molecule with heterocyclic and other fragments (usually together with variations of substituents in the phenyl rings and in some cases with the replacement of phenyl rings with aromatic heterocycles or their analogs).[1—5] In the course of transformation of such compounds, the distance between the aromatic groups frequently remains the same as in the starting molecule. However, lately a tendency to the development of combretastatin analogs with longer linkers becomes increasingly popular: there are literature examples of several successful types of such compounds with high cytotoxicity against different lines of tumor cells (see, for example, Refs 6—8). In this work, we suggested structures (**A**) as analogs of CA-4, in which the aromatic groups are connected with an elongated linker as compared to that in the starting molecule. This linker is a combination of a heterocyclic fragment and a double bond, which was not studied earlier for this purpose.



A

Initially, we studied easily synthetically available (Scheme 1) 5-arylmethylideneoxazol-4-ones **5**—**9**. An au-

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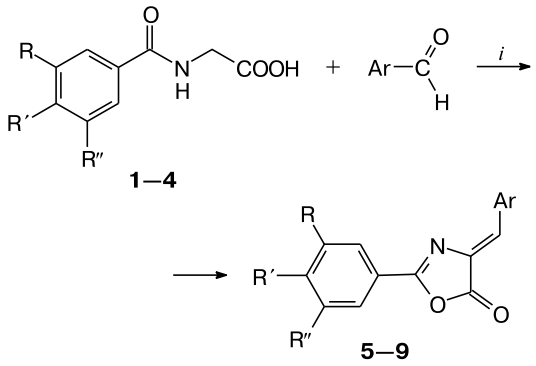
tomated docking of these compounds into the colchicine binding site using the CLC Drug Discovery Workbench program showed that in the case of *Z*-configuration of the C=C double bond, their position is close enough to that of CA-4 except a slight "shift" of ring B (Fig. 1).

Scheme 1



Reagents and conditions: *i*. Ac_2O, CH_3COOK, heating.

Compound	R	R′	R″
1, **5**	OMe	OMe	OMe
2, **6**	H	Me	H
3, **7**, **8**	H	F	H
4, **9**	H	H	H

Product	Ar	Yield (%)
5	4-MeO-C_6H_4	27
6	4-MeO-C_6H_4	46
7	4-MeO-C_6H_4	22
8	3,4-$(MeO)_2$-C_6H_3	21
9	Ph	99

The choice of substituents in the aryl fragments of oxazolones **5**—**8** was based on both the results of the computer molecular modeling (the scoring function values) and the literature data on the bioisostericity of *para*-fluorophenyl and 3,4,5-trimethoxyphenyl groups in some active analogs of CA-4 with an elongated linker.[8]

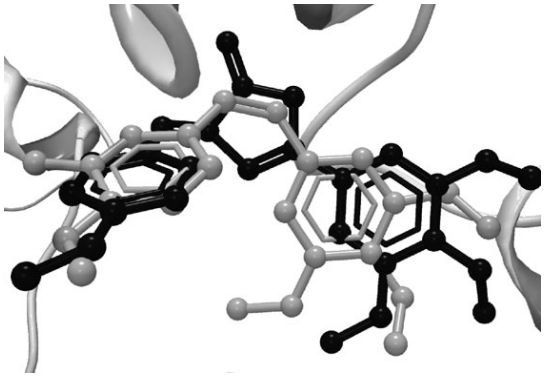


Fig. 1. Position of structure **5** in the colchicine domain of tubulin (for comparison, a CA-4 molecule is shown in light; hydrogen atoms are omitted).

Compound **9** without substituents in the aryl fragments was obtained as a control.

5-Arylmethylideneoxazol-4-ones **5**—**9** were synthesized from substituted hippuric acids **1**—**4**, which were obtained from aromatic carboxylic acyl halides and glycine according to the known procedures[9—13] (in the case of 3,4,5-trimethoxyhippuric acid **1**, 3,4,5-trimethoxybenzoic acid was additionally synthesized by trimethylation of gallic acid with excess of dimethyl sulfate[13]). *N*-Acylglycines **1**—**4** were involved in the condensation reaction with aromatic aldehydes, using potassium acetate as a base (see Scheme 1), which increased the yield of the desired *Z*-isomer[14] (the signal for the vinyl proton is in the region δ 7.1—7.3). A wide range of the target compound yields (see Scheme 1) is apparently explained by their different solubilities, as well as by a possibility of side hydrolysis reaction of oxazolone **5** during column chromatography.

The testing of compounds **5**—**9** on the human epithelial lung carcinoma cells A549 in a standard colorimetric test with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolyl bromide (MTT)[15] showed that their cytotoxicity is low (EC_{50} > 50 μmol L^{-1}) and does not depend on the type of substituents in the aryl rings. This can possibly result from the mentioned above and, by all accounts, unfavorable "shift" of ring B in 5-arylmethylidenoxazol-4-ones (see Fig. 1).

To confirm this conclusion, we synthesized another two compounds related to the suggested structural type **A**, namely, *N*-methyl-3,5-dihydro-4*H*-imidazol-4-ones **10** and **11** with different positions of rings B, according to the computer molecular modeling data (Fig. 2, *a*, *b*). As it is seen, in the structure **10** ring B is closer to that in CA-4, while in the structure **11**, it is "shifted" as compared to rings B in the starting molecule and in 2-aryl-5-arylmethylidenoxazol-4-ones.

The synthesis of compounds **10** and **11** was accomplished by reflux of oxazolones in solution of methylamine in aqueous ethanol in the presence of potassium carbonate as a base (Scheme 2).[16] The ^1H NMR spectra of final compounds exhibit the only signal for the vinyl proton in the region δ 6.6 and 7.04, that confirm the presence of only one geometric isomer (*Z*-isomer[17]). A singlet for the protons of the CH_3N group is observed at δ 3.65 and 3.45, respectively.

The examination of compounds **10** and **11** in the MTT-test showed that imidazolone **11** is inactive (EC_{50} > 50 μmol L^{-1}), while imidazolone **10** exhibits a noticeable cytotoxicity (EC_{50} = 6±0.8 μmol L^{-1}) against the A549 cell culture. These data indirectly confirm a conclusion that the activity of compounds of this type is sensitive to the insignificant changes in the position of ring B. (5*Z*)-5-[(4-Methoxyphenyl)methylidene]-3-methyl-2-(4-methylphenyl)-3,5-dihydro-4*H*-imidazol-4-one obtained in this work can become a lead compound in further search

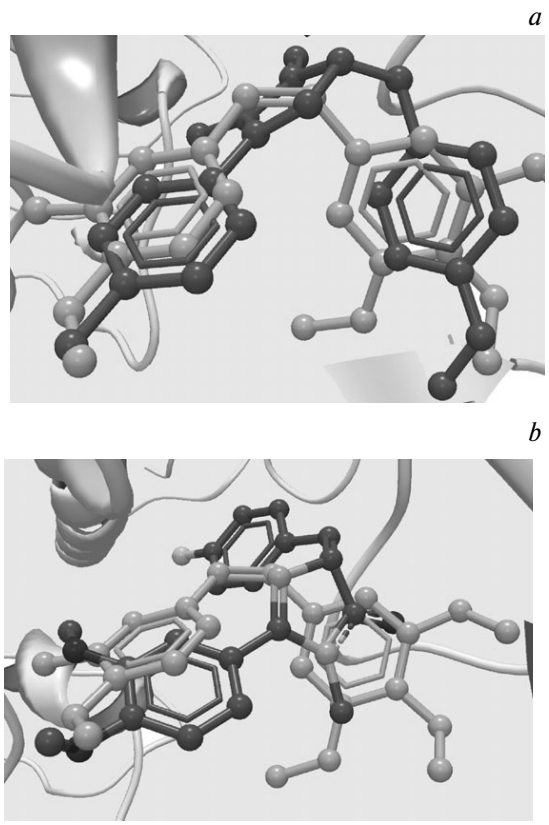
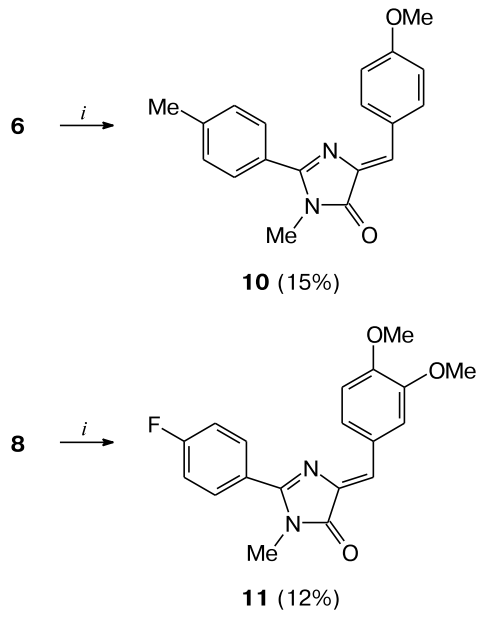


Fig. 2. Position of structures **10** (*a*) and **11** (*b*) in the colchicine domain of tubulin (for comparison, a CA-4 molecule is shown in light; hydrogen atoms are omitted).

Scheme 2



Reagents and conditions: *i*. $MeNH_2$, K_2CO_3, heating.

for more efficient analogs of combretastatin as potential antitumor agents.

Experimental

Reaction progress and purity of compounds were monitored by TLC on Silufol-UV 254 plates. Chromatographic separation was carried out on columns with Acros silica gel (40—60 μm). ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 and 100 MHz, respectively) at 28 °C. Chemical shifts are given relative to the residual signals of the solvent. Elemental analysis was performed on a Vario micro cube CHN-analyzer. IR spectra were recorded on an IR-200 ThermoNicolet spectrophotometer in KBr pellets. Electron ionization mass spectra were obtained on a Finnigan MAT SSQ 7000 GLC-MS spectrometer (ionization energy 70 eV, an OV-I quartz capillary column, 25 m). Automated docking in the 3D model of tubulin complex with *N*-deacetyl-*N*-(2-mercaptoacetyl)colchicine (PDB ID: 1SA0) was performed using the CLC Drug Discovery Workbench program (Version 1.5): Evaluation license (2014).

(4*Z*)-4-[(4-Methoxyphenyl)methylidene]-2-(3,4,5-trimethoxyphenyl)-1,3-oxazol-5(4*H*)-one (5). A mixture of 4-methoxybenzaldehyde (0.285 g, 2.1 mmol), acid **1** (0.5 g, 2.1 mmol), and potassium acetate (0.21 g, 2.1 mmol) in $(CH_3CO)_2O$ (5 mL) was refluxed with stirring for 12 h. The solvent was evaporated, the residue was subjected to chromatography (eluent ethyl acetate—light petroleum ether (40—70 °C), 1 : 1). Dried *in vacuo* of an oil pump. Compound **5** (0.21 g, 27%) was obtained as brown oily liquid. $R_f = 0.18$. ^1H NMR ($CDCl_3$), δ: 7.63 (s, 2 H, Ar); 7.45 (d, 2 H, Ar, $J = 8.8$ Hz); 7.27 (s, 1 H, HC=); 6.98 (d, 2 H, Ar, $J = 8.8$ Hz); 3.88 (s, 3 H, CH_3O); 3.80 (s, 9 H, CH_3O). MS, *m*/*z* (I_{rel} (%)): 369.4 $[M]^+$ (10). Found (%): C, 64.98; H, 5.09; N, 3.78. $C_{20}H_{19}NO_6$. Calculated (%): C, 65.04; H, 5.15; N, 3.79.

(4*Z*)-4-[(4-Methoxyphenyl)methylidene]-2-(4-methylphenyl)-1,3-oxazol-5(4*H*)-one (6). A mixture of anisaldehyde (0.37 mL, 3 mmol), 4-methylhippuric acid (0.75 g, 3 mmol), and potassium acetate (0.3 g, 3 mmol) in acetic anhydride (5 mL) was refluxed for 2 h. A precipitate formed was filtered and washed with ethanol to obtain compound **6** (0.389 g, 46%), yellow crystals, m.p. 186—187 °C (Ref. 18: m.p. 186 °C). ^1H NMR ($CDCl_3$), δ: 8.18 (d, 2 H, Ar, $J = 7.8$ Hz); 8.04 (d, 2 H, Ar, $J = 8.4$ Hz); 7.31 (d, 2 H, Ar, $J = 7.8$ Hz); 7.24—7.18 (m, 3 H, Ar, HC=); 3.88 (s, 3 H, CH_3O); 2.44 (s, 3 H, CH_3). IR, ν/cm^{-1}: 1792 (C=O), 1651 (C=N). Found (%): C, 73.66; H, 5.19; N, 4.62. $C_{18}H_{15}NO_3$. Calculated (%): C, 73.72; H, 5.12; N, 4.78.

(4*Z*)-2-(4-Fluorophenyl)-4-[(4-methoxyphenyl)methylidene]-1,3-oxazol-5(4*H*)-one (7) was obtained according to the procedure described earlier[19] using potassium acetate. ^1H NMR spectrum agrees with that reported in the literature (see Ref. 20, supporting information).

(4*Z*)-4-[(3,4-Dimethoxyphenyl)methylene]-2-(4-fluorophenyl)-1,3-oxazol-5(4*H*)-one (8). A mixture of 3,4-dimethoxybenzaldehyde (0.3 g, 2.5 mmol), 4-fluorohippuric acid (0.5 g, 2.5 mmol), and potassium acetate (0.25 g, 2.5 mmol) in $(CH_3CO)_2O$ (5 mL) was refluxed for 3 h with stirring. A precipitate formed was filtered and recrystallized from ethanol to obtain compound **8** (0.19 g, 21%), yellow crystals, m.p. 188—190 °C. ^1H NMR ($CDCl_3$), δ: 8.12—8.15 (m, 3 H, Ar); 7.57 (d, 1 H, Ar, $J = 8.4$ Hz); 7.18—7.23 (m, 3 H, Ar, HC=); 6.94 (d, 1 H, Ar, $J = 8.4$ Hz);

3.01 (s, 3 H, CH_3); 3.96 (s, 3 H, CH_3). Found (%): C, 60.98; H, 4.79; N, 3.93. $C_{18}H_{14}NO_4F \cdot 1.5H_2O$. Calculated (%): C, 61.02; H, 4.84; N, 3.95.

(4*Z*)-2-Phenyl-4-(phenylmethylidene)-1,3-oxazol-5(4*H*)-one (9) was obtained according to the procedure described earlier[19] using potassium acetate. ^{1}H NMR spectrum agrees with that reported in the literatures (see Ref. 21, supporting information).

(5*Z*)-5-[(4-Methoxyphenyl)methylidene]-3-methyl-2-(4-methylphenyl)-3,5-dihydro-4*H*-imidazol-4-one (10). A 40% aqueous solution of methylamine (5 mL) and potassium carbonate (0.1 g, 0.7 mmol) were added to a solution of oxazolone **6** (0.2 g, 0.7 mmol) in ethanol with stirring, the mixture was refluxed for 8 h with stirring. The solvent was evaporated, the residue was subjected to chromatography (eluent ethyl acetate—light petroleum ether (40—70 °C), 1 : 3, then ethyl acetate) to obtain compound **10** (0.031 g, yield 15%), orange oily liquid, R_f = 0.53 (ethyl acetate—light petroleum ether, 1 : 3). ^{1}H NMR (DMSO-d_6), δ: 7.85 (d, 2 H, Ar, J = 8.0 Hz); 7.51 (d, 2 H, Ar, J = 7.9 Hz); 6.90—7.05 (m, 4 H, Ar); 6.60 (s, 1 H, HC=); 3.89 (s, 3 H, CH_3O); 3.50 (s, 3 H, CH_3N); 2.13 (s, 3 H, CH_3). MS, m/z (I_{rel} (%)): 307.30 $[M + H]^+$ (60). Found (%): C, 74.47; H, 5.87; N, 9.12. $C_{18}H_{15}NO_3$. Calculated (%): C, 74.49; H, 5.92; N, 9.14.

(5*Z*)-5-[(3,4-Dimethoxyphenyl)methylidene]-2-(4-fluorophenyl)-3-methyl-3,5-dihydro-4*H*-imidazol-4-one (11) was synthesized similarly to compound **10** from oxazolone **8** (0.15 g, 0.4 mmol), 40% aqueous solution of methylamine (5 mL), and potassium carbonate (0.1 g, 0.7 mmol) to obtain compound **10** (0.02 g, 12%), orange oily liquid, R_f = 0.6 (ethyl acetate—light petroleum ether, 1 : 3). ^{1}H NMR (DMSO-d_6), δ: 7.81 (d, 2 H, Ar, J = 8.8 Hz); 7.48 (d, 1 H, Ar, J = 8.2 Hz); 7.38 (dd, 2 H, Ar, J_1 = 8.8 Hz, J_2 = 1.0 Hz); 7.12 (s, 1 H, Ar); 7.04 (s, 1 H, HC=); 7.00 (d, 1 H, Ar, J = 8.2 Hz); 3.83 (s, 3 H, OCH_3); 3.78 (s, 3 H, OCH_3); 3.45 (s, 3 H, NCH_3). Found (%): C, 66.99; H, 5.01; N, 8.22. $C_{18}H_{15}NO_3$. Calculated (%): C, 67.05; H, 5.03; N, 8.23.

MTT-test on cytotoxicity was carried out on human epithelial lung carcinoma cells (line A-549, CCL-185) according to the procedures described in the works.[22,23]

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