Synthesis and antineoplastic properties of (1H-1,2,3-triazol-1-yl)furazans*

A. S. Kulikov, a M. A. Epishina, a L. V. Batog, a V. Yu. Rozhkov, a N. N. Makhova, a L. D. Konyushkin, a M. N. Semenova, b and V. V. Semenov a

aN. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation. Fax: +7 (499) 135 5328. E-mail: mnn@ioc.ac.ru

bN. K. Kol’tsov Institute of Developmental Biology, Russian Academy of Sciences, 26 ul. Vavilova, 119334 Moscow, Russian Federation. Fax: +7 (499) 135 8012

A method of 3-amino-4-[5-aryl(heteroaryl)-1H-1,2,3-triazol-1-yl]furazan synthesis was optimized. Condensation of these compounds with 2,5-dimethoxytetrahydrofuran resulted in a series of previously unknown 4-[5-aryl(heteroaryl)-1H-1,2,3-triazol-1-yl]-3-(pyrrol-1-yl)furazans. All target compounds were evaluated for both antimitotic microtubule destabilizing effect in a phenotypic sea urchin embryo assay and cytotoxicity in a panel of 60 human cancer cell lines. Pyrrolyl derivatives of triazolylfurazans were determined as antiproliferative compounds. The most potent microtubule targeting compounds 7a and 7e are of interest for further trials as antineoplastic agents.

Key words: azidofurazans, 1,3-dicarbonyl compounds, 1,3-dipolar cycloaddition, 2,5-dimethoxytetrahydrofuran, 3-amino(pyrrol-1-yl)-4-[5-aryl(hetaryl)-1H-1,2,3-triazol-1-yl]-furazans, antineoplastic activity, sea urchin embryos.

Five-membered heterocycles are frequently used in the synthesis of antimitotics that was studied in detail1 for the analogs of natural compound combretastatin A-4 (CA-4). A water-soluble phosphorylated prodrug of CA-4 is currently undergoes clinical trials in the USA as antitumor agent.2,3 An introduction of 1,2,3-triazole (1,2,3-triazolocombretastatin)4,5 and furazan (combretafurazan)6 rings into combretastatin framework was regarded as a non-isomerizable and metabolically stable bioisosteric replacement of the double bond in cis-stilbenes allowing the synthesis of new promising anticancer compounds.

Compared to combretastatin, combretafurazan is a more potent cytotoxic compound in vitro against neuroblastoma cells, yet maintaining similar pharmacokinetic properties.6 1,2,3-Triazole-bridged combretastatin analog7-9 exhibits both strong cytotoxicity against ovarian cancer cells and vascular disrupting activity in tumors. Moreover, this compound is more water-soluble than combretafurazan.

Water-soluble biologically active compounds containing both cycles, e.g., (1,2,3-triazol-1-yl)furazans 1, exhibiting other mechanisms of action were synthesized. Thus, compounds with the (1,2,3-triazol-1-yl)furazan moieties inhibit glycogen synthase kinase (GSK-3), a target in the treatment of Alzheimer’s disease and type 2 diabetes.7 Other analogs of (1,2,3-triazol-1-yl)furazans inhibit the SARS CoV Mpro cysteine protease, an important enzyme responsible for the intracellular replication of severe acute respiratory syndrome coronavirus.8 Several (1,2,3-triazol-1-yl)furazan derivatives selectively stimu-

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late NO-dependent activation of soluble guanylate cyclase (sGC). \(^{3b}\)

In the present work we aimed to study a series of (1,2,3-triazol-1-yl)furazans 1 as potential antineoplastic agents, since these compounds can be synthesized by well elaborated methods. \(^{9—15}\) Synthesis of these compounds involved 1,3-dipolar cycloaddition of azidofurazans 2 to various dipolarophiles, e.g., acetylenes, morpholinonitroethylene, or compounds with the activated methylene group, e.g., activated nitriles and 1,3-dicarbonyl compounds. The disadvantage of majority of these methods is the formation of 1,2,3-triazole regioisomers. Depending on the type of the substituents, dipolarophiles add differently to the azidofurazans yielding isomeric 4,5- or 5,4-derivatives (Scheme 1).

![Scheme 1](image)

The regioselective cycloaddition of aroylacetic esters 3a—g were involved in the cyclocondensations with aminoazidofurazan 2a under conditions developed earlier. \(^{10}\) The \(^1\)H and \(^13\)C NMR spectra of synthesized triazolylfurazans 4a—g indicated formation of single regioisomer in high yield; no signals for the second possible regioisomer were detected. The spectral characteristics also indicated high purity of the crude products. Therefore, unpurified esters 4a—g were hydrolyzed to the corresponding acids 5a—g, which also without further purification were subsequently thermally decarboxylated to target 3-amino-4-[5-aryl(hetaryl)-1H-1,2,3-triazol-1-yl]furazans 6a—g in high yields. Thus, we developed a preparative procedure for the synthesis of triazolylfurazans 6a—g without purification of the intermediates (Scheme 2).

To transform the amino group of triazolylfurazans 6 into the pyrrole ring, compounds 6a—f and 6h were involved in the Clauson—Kaas pyrrole synthesis with 2,5-dimethyltetrahydrofuran. The reaction was carried out in refluxing acetic acid. \(^{14}\) Pyrrole-containing triazolylfurazans 7a—f,h were obtained in 64—98% yields (Scheme 3).

![Scheme 2](image)

**Reagents and conditions:** \(i\). MgCO\(_3\), EtOH, reflux, 8—10 h; \(ii\). NaOH, H\(_2\)O, reflux, 1 h; \(iii\). AcOH, reflux, 45 min.
The biological activity of seven triazolylfurazan derivatives bearing amino groups 6a—d,g,i, ester 4c (a precursor of compound 6c), and seven pyrrole derivatives 7a—f,h was studied. The initial trials were carried out on the sea urchin embryos widely used as a model in screening for compounds with antiproliferative effect. Reagents and conditions: AcOH, reflux, 1 h.

**Biological trials**

The biological activity of seven triazolylfurazan derivatives bearing amino groups 6a—d,g,i, ester 4c (a precursor of compound 6c), and seven pyrrole derivatives 7a—f,h was studied. The initial trials were carried out on the sea urchin embryos widely used as a model in screening for compounds with antiproliferative effect. 

Recently a simple and efficient phenotypic sea urchin embryo assay has been developed. The assay allows identification of compounds with antiproliferative properties and provides information about the mechanism of antimitic activity. Specific changes of sea urchin eggs at concentration of 50 nmol L⁻¹ caused the sea urchin embryo swimming suggesting the antitubulin mechanism of action, namely, the ability to destabilize microtubules. Apparently, compound 7a—f,h exhibited similar mechanism of action. Although, compound 7a failed to affect the sea urchin embryo swimming, the arrested eggs acquired tuberculate shape typical of microtubule destabilizers. The pyrrole ring was shown to be essential for antiproliferative effect, since the related structures 6 containing the amino group instead of the pyrrole ring were inactive. It is worth noting that the increase in the number of methoxy groups in the benzene ring (compounds 6a—d) resulted in reduction of the antimitotic properties. In this respect, triazolylfurazans 7 is distinguished from the known analogs of plant antimitotics combretastatin and podophyllotoxin interacting with the colchicine site of tubulin. Specifically, trimethoxybenzene

**Table 1. Antiproliferative activity of compounds 4c, 6a—d,g,i and 7a—f,h**

| Compound | Sea urchin embryo, EC/μmol L⁻¹ | Inhibition of human cancer cell growth (%) |
|----------|-------------------------------|------------------------------------------|
|          | Cleavage alteration | Cleavage arrest | Embryo spinning |
| 4c       | >4                        | >4                          | >4                        | 105.15                |
| 6a       | 4                         | >4                          | >4                        | 103.97                |
| 6b       | >4                        | >4                          | >4                        | 95.88                 |
| 6c       | >4                        | >4                          | >4                        | 101.33                |
| 6d       | >4                        | >4                          | >4                        | 100.34                |
| 6g       | >4                        | >4                          | >4                        | 102.88                |
| 6i       | >4                        | >4                          | >4                        | 103.66                |
| 7a       | 0.05                      | 0.5                         | >5                        | (0.389)               |
| 7b       | 0.5                       | >5                          | >5                        | 50.83                 |
| 7c       | 1                         | >5                          | >5                        | 98.26                 |
| 7d       | 5                         | >5                          | >4                        | —                      |
| 7e       | 0.05                      | 0.5                         | 5                         | (0.295)               |
| 7f       | 4                         | >4                          | >4                        | 89.97                 |
| 7h       | >4                        | >4                          | >4                        | 99.63                 |

Note. The effect of compounds on the sea urchin embryos was studied according to the described method. Repeated measurements showed no differences in effective threshold concentration. Inhibition of human cancer cell growth (percentage to control) was determined for the concentration of the tested compound of 10 μmol L⁻¹. GI₅₀ is compound concentration required for 50% cell growth inhibition. The average GI₅₀ values and percent of inhibition of cancer cell growth for 60 human cancer cell lines (NCI60 screen, http://dtp.cancer.gov) are given.

* Compound 6i was kindly provided for the studies by Chemical Block Ltd (www.chemblock.com).

**Scheme 3**

![Scheme 3](image)

**Table 1. Antiproliferative activity of compounds 4c, 6a—d,g,i and 7a—f,h**

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* Video illustrations of sea urchin embryo swimming are presented at http://www.chemblock.com.
Antineoplastic properties of triazolylfurazans

**Fig. 1.** Effect of triazolylfurazans on the sea urchin *Paracentrotus lividus* embryo development with compound 7e as an example. (a) Intact blastula. (b) and (c) Typical developmental alterations caused by compound 7e at concentration of 0.1 μmol L⁻¹ (b, abnormal cleavage) and 0.5 μmol L⁻¹ (c, arrested tuberculate egg). The observations were carried out 6 h after fertilization. The average embryo diameter was 115 μm.

derivatives of combretastatin and podophyllotoxin exhibit the strongest antimitotic activity.19,20

According to the data of the National Cancer Institute (NCI) of USA, compounds 7a and 7e inhibited cancer cell growth at relatively low concentrations (GI₅₀ = 389 and 295 nmol L⁻¹, respectively). These compounds were referred to the biological expert committee of NCI as promising for further studies. Leukemia SR cells (7a), melanoma MDA-MB-435 cells (7a and 7e), and the colon cancer cells (7e) were the most sensitive to triazolylfurazans 7a and 7e. The "doze—effect" curves for seven colon cancer cell lines exposed to compound 7e are given in Fig. 2.

In summary, the preparative synthesis of furazans 6 by 1,3-dipolar cycloaddition of azidoaminofurazan 2a to aroyl(hetaroyl)acetates 3a—g followed by further modification was developed. The procedure does not require purification of the intermediates. Subsequent Clauson—Kaas condensation of synthesized triazolylfurazans 6 with dimethoxetylhydrofururan yielded a series of 4-[5-aryl-(hetaryl)-1H-1,2,3-triazol-1-yl]- (3-pyrryl-1-yl)furazans 7. The antiproliferative properties of both types of compounds were evaluated using the sea urchin embryo assay. It was found that the amino derivatives of triazolylfurazans 6 failed to affect cell division. However, their analogs 7 bearing the pyrrole ring exhibited moderate antimitotic activity. Two compounds, 7a and 7c, were referred to the NCI biological expert committee as promising compounds for further trials.

**Experimental**

NMR spectra were recorded on Bruker WM-250 (1H, 250 MHz) and Bruker AM-300 (13C, 75.5 MHz) spectrometers. Chemical shifts are given in the δ scale relative to Me₄Si (internal standard). Mass spectra were obtained on a Varian MAT CH 6 instrument (EI, 70 eV). Thin-layer chromatography was performed on Silufol UV-254 plate (elution with CHCl₃), spots were visualized under UV light. Elemental analyses were carried out on a Perkin—Elmer 2400 CHN analyzer.

Ethyl aroylacettes with 4-methoxyphenyl- (3a), 3,4-di-methoxypyhenyl- (3b), 3,4,5-trimethoxypyhenyl- (3c), 4-fluorophenyl substituents (3f) were commercially available (Aldrich). 4-Azidofurazan-3-amine (2a), ethyl aroylacettes with 3,4-methyleneoxyphenyl- (3d), 4-ethoxyphenyl- (3e), and 2-thienyl substituents (3g)21 and 4-[5-(4-chlorophenyl)-1H-1,2,3-triazol-1-yl]furazane-3-amine (6h)15 were synthesized by the known procedures.

**Synthesis of ethyl 1-(4(4-aminofurazan-3-yl))-5-R-1H-1,2,3-triazole-4-carboxylates 4a—g (general procedure).** A mixture of 4-azidofurazan-3-amine 2a (0.88 g, 7 mol), aroylacette 3a—g (7 mmol), and MgCO₃ (0.34 g, 4 mmol) in ethanol (20 mL) was refluxed for 8—10 h (until complete consumption of 2a, TLC monitoring). The reaction mixture was filtered hot, the solvent was evaporated in vacuo. The precipitate was filtered off, washed with cold EOH, and dried in air.

**Ethyl 1-(4(4-aminofurazan-3-yl))-5-(4-methoxyphenyl)-1H-1,2,3-triazole-4-carboxylate (4a).** The yield was 96%, m.p.
184—185 °C, Rf 0.57. Found (%): C, 50.76; H, 4.40; N, 25.33. C14H14N6O6. Calculated (%): C, 49.68; H, 4.65; N, 24.41.

Ethyl 1-(4-aminofurazan-3-yl)-5-(3,4-dimethoxyphenyl)-1,2,3-triazole-4-carboxylate (4a). The yield was 82%, m.p. 192—194 °C, Rf 0.62. Found (%): C, 50.19; H, 4.34; N, 23.22. C13H13N6O6. Calculated (%): C, 50.00; H, 4.48; N, 23.22. MS, m/z (Irel (%)): 360 [M]+ (89); 255 [M – Me]+ (25); 286 (29); 288 (15); 325 [ArCOCCH3]+ (19) (71); 170 (100); 177 (71); 176 (15). 1H NMR (DMSO-d6), δ: 1.25 (6 H, Me, 3J = 4.2 Hz); 3.82, 3.86 (2 s, 2 H, OMe); 6.56 (2 s, 2 H, Ar, 3J = 4.0 Hz); 6.86—6.99 (3 H, Ar). 13C NMR (DMSO-d6), δ: 55.27 (OMe); 113.84; 120.65; 134.22; 139.57; 143.13 (Me); 60.98 (CH2); 102.70; 108.15; 119.85; 136.55; 139.12; 149.81; 145.13; 153.30; 157.72 (CNH2); 160.79 (CO); 163.12 (CO).

Ethyl 1-(4-aminofurazan-3-yl)-5-(3,4,5-trimethoxyphenyl)-1,2,3-triazole-4-carboxylate (4b). The yield was 94%, m.p. 189—192 °C, Rf 0.64. Found (%): C, 50.19; H, 4.34; N, 23.22. C13H13N6O6. Calculated (%): C, 49.68; H, 4.65; N, 24.41. MS, m/z (Irel (%)): 360 [M]+ (89); 255 [M – Me]+ (25); 286 (29); 288 (15); 325 [ArCOCCH3]+ (19) (71); 170 (100); 177 (71); 176 (15). 1H NMR (DMSO-d6), δ: 1.25 (6 H, Me, 3J = 4.2 Hz); 3.82, 3.86 (2 s, 2 H, OMe); 6.56 (2 s, 2 H, Ar, 3J = 4.0 Hz); 6.86—6.99 (3 H, Ar). 13C NMR (DMSO-d6), δ: 55.27 (OMe); 113.84; 120.65; 134.22; 139.57; 143.13 (Me); 60.98 (CH2); 102.70; 108.15; 119.85; 136.55; 139.12; 149.81; 145.13; 153.30; 157.72 (CNH2); 160.79 (CO); 163.12 (CO).
Antiproteolytic properties of triazolylfurazans.

**Antiproliferative properties of triazolylfurazans.**

The yield was 90%, m.p. 176—177 °C. Found (%): C, 45.35; H, 2.42; N, 26.50.

C<sub>3</sub>H<sub>2</sub>N<sub>2</sub>O<sub>2</sub>. Calculated (%): C, 45.58; H, 2.43; N, 26.59.

1H NMR (DMSO-d<sub>6</sub>), δ 7.13 (s, 2 H, NH<sub>2</sub>); 6.93, 7.28 (both d, 2 H each, Ar, J<sub>Ar</sub> = 8.2 Hz); 6.08 (s, 2 H, CH<sub>2</sub>); 6.67 (s, 2 H, NH<sub>2</sub>); 7.74, 7.92 (both d, 2 H each, Ar, J<sub>Ar</sub> = 8.0 Hz); 12.96 (br.s, 1 H, OH).

13C NMR (DMSO-d<sub>6</sub>), δ 172.32 (Me); 60.40 (CH<sub>2</sub>); 111.95; 114.90; 131.17; 137.27; 142.04; 143.52; 153.88; 160.46 (CNH<sub>2</sub>); 161.55 (CO).

1-(4-Aminofurazan-3-yl)-5-(2-fluorophenyl)-1,2,3-triazole-4-carboxylic acid (5e). The yield was 53%, m.p. 192—193 °C, R<sub>f</sub> 0.13. Found (%): C, 52.41; H, 4.59; N, 31.02. C<sub>12</sub>H<sub>9</sub>N<sub>5</sub>O<sub>3</sub>. Calculated (%): C, 52.94; H, 4.44; N, 30.87. MS, m/z (J<sub>Ar</sub>) (272 [M+]: 67); 244 [M – N<sub>2</sub>] (62); 186 (14); 172 (19); 160 (100).

1H NMR (DMSO-d<sub>6</sub>), δ 8.08 (s, 2 H, CH<sub>2</sub>); 6.67 (s, 2 H, NH<sub>2</sub>); 7.64, 7.02 (both d, 2 H, in Ar, J<sub>Ar</sub> = 7.5 Hz); 7.00 (1 H, CH in Ar); 8.22 (s, 1 H, triazole ring). 13C NMR (DMSO-d<sub>6</sub>), δ 101.83 (CH<sub>2</sub>); 108.46; 118.26; 137.76; 147.33; 147.53; 150.00 (CNH<sub>2</sub>); 160.65 (CO); 164.26.

### 4-(5-Ethoxyphenyl)-1,1,2,3-triazol-1-yl]furazan-3-amine (6a). The yield was 110%, m.p. 174—175 °C. Found (%): C, 39.05; H, 2.28; N, 30.12. C<sub>8</sub>H<sub>5</sub>N<sub>6</sub>O<sub>2</sub>. Calculated (%): C, 38.85; H, 2.17; N, 30.20. MS, m/z (J<sub>Ar</sub>) (278 [M+]: 22); 234 [M – CO<sub>2</sub>] (24); 206 (14); 125 (25); 134 (31); 122 (100).

1H NMR (DMSO-d<sub>6</sub>), δ 8.43 (s, 2 H, NH<sub>2</sub>); 7.13 (m, 1 H, C(4) thiophene ring); 7.44 (d, 1 H, C(3) thiophene ring); 7.73 (d, 1 H, C(5) thiophene ring). 1J<sub>2H</sub> = 5.2 Hz.

**Synthesis of 3-amino-4-(5-R-1,2,3-triazol-1-yl]furazan**

6 general procedure. A solution of carboxylic acid 5 (general procedure).

### 4-[(4-Methoxyphenyl)-1,1,2,3-triazol-1-yl]furazan-3-amine (6a). The yield was 76%, m.p. 166—167 °C, R<sub>f</sub> 0.10. Found (%): C, 51.22; H, 3.81; N, 32.43. C<sub>4</sub>H<sub>6</sub>N<sub>2</sub>O<sub>2</sub>. Calculated (%): C, 51.16; H, 3.90; N, 32.54. MS, m/z (J<sub>Ar</sub>) (258 [M+]: 7); 230 [M – N<sub>2</sub>] (12); 158 (10); 146 (100).

1H NMR (DMSO-d<sub>6</sub>), δ 3.79 (3 H, OMe); 6.65 (2 H, NH<sub>2</sub>); 7.05, 7.35 (both d, 4 H in Ar, J<sub>Ar</sub> = 8.0 Hz); 8.23 (1 H, triazole ring). 13C NMR (DMSO-d<sub>6</sub>), δ 55.30 (OMe); 114.55; 116.78; 129.68; 132.60; 139.85; 143.13; 153.19; 160.49.
[M(Cl(35))]+ (18); 268; 424 [M(Cl(37))]+ (4); 240 [M(Cl(35))−N(\text{Cl})]+ (11); 196 (12); 170 (8); 168 (23); 150 (41); 156 (100).

Found (%): C, 59.80; H, 4.29; N, 26.19. C14H14N6O3 Cl2. Calculated (%): C, 59.62; H, 4.38; N, 26.07. MS, m/z (Jrel(%)): 322 [M]+ (32); 294 [M−N]+ (53); 264 (21); 236 (38); 209 (52); 144 [Ar&Cl]+ (28); 132 (100). 1H NMR (DMSO-d6), δ: 1.31 (t, 3 H, Me, J = 7.0 Hz); 4.05 (q, 2 H, CH2, J = 7.0 Hz); 6.35 (s, 2 H, C(3) and C(4) of pyrrole ring); 6.83 (s, 2 H, C(2) and C(5) of pyrrole ring); 7.48, 7.52 (d, 2 H each, Ar, J = 8.4 Hz); 8.37 (s, 1 H, triazole ring). 13C NMR (DCl3), δ: 139.00 (Me); 62.26 (OCH3); 113.25; 114.72; 117.33; 130.11; 133.18; 142.52; 143.92; 148.70; 161.08.

4-[5-(4-Fluorophenyl)-1H,1,2,3-triazol-1-yl]-3-(pyrrol-1-yl)furazan (7f). The yield was 80%, m.p. 122—123 °C, Rf 0.52. Found (%): C, 56.59; H, 2.98; N, 28.19. C14H9F2N2O. Calculated (%): C, 56.76; H, 3.06; N, 28.37. MS, m/z (Jrel(%)): 296 [M]+ (17); 268 [M−N]+ (80); 267 (24); 238 (51); 211 (100); 185 (58); 146 (28); 134 (74); 126 (76); 120 [Ar&Cl]+ (64); 107 (79). 1H NMR (DMSO-d6), δ: 3.85 (s, 2 H, C(3) and C(4) of pyrrole ring); 6.83 (s, 2 H, C(2) and C(5) of pyrrole ring); 7.28, 7.49 (both d, 2 H each, Ar, J = 8.0 Hz); 8.40 (s, 1 H, triazole ring). 13C NMR (DCl3), δ: 115.39; 117.16; 132.40; 137.73; 144.18; 146.82; 146.95; 162.29; 164.80.

4-[5-(4-Chlorophenyl)-1H,1,2,3-triazol-1-yl]-3-(pyrrol-1-yl)furazan (7h). The yield was 76%, m.p. 97—98 °C, Rf 0.44. Found (%): C, 53.44; H, 3.01; N, 27.03. C15H10ClN2O. Calculated (%): C, 53.77; H, 2.90; N, 26.87. MS, m/z (Jrel(%)): 314 [M(Cl(35))+ (3); 312 [M(Cl(37))+ (13); 286 [M(Cl(35))−N]+ (18); 284 [M(Cl(35))−N]+ (53); 256 (21); 254 (40); 229 (11); 227 (36); 219 (46); 192 (96); 167 (37); 150 (53); 138 (13); 136 (33). 1H NMR (DMSO-d6), δ: 3.62 (s, 2 H, C(3) and C(4) of pyrrole ring); 6.84 (s, 2 H, C(2) and C(5) of pyrrole ring); 7.48, 7.51 (both d, 2 H each, Ar, J = 8.6 Hz); 8.44 (s, 1 H, triazole ring). 13C NMR (DCl3), δ: 143.92; 148.70; 161.08.

Study of antiproliferative activity of compounds using a sea urchin embryo assay.18 The trials were carried out in the biological laboratory of N. K. Kol’tsov Institute of Developmental Biology of RAS in Cyprus. Adult sea urchins, Paracentrotus lividus L. (Echinidae), were collected from the Mediterranean Sea on the Cyprus coast and kept in an aerated seawater tank. Gametes were obtained by intracoelomic injection of 0.5 M KCl (1—2 mL). Eggs were washed with filtered seawater and fertilized by adding drops of diluted sperm. Embryos (600—2000 mL−1) were cultured in filtered seawater at room temperature (18—23 °C) in six-well culture plates.

Stock solutions of compounds were prepared in DMSO or 95% aqueous ethanol, the maximal studied concentrations of the compounds depended on their solubility. The solubility of compounds in the solvents and seawater was controlled under microscope.

Compound treatment was carried out at the following developmental steps: (1) fertilized eggs, 8—15 min after fertilization; (2) hatched swimming blastulae, 8.5—10 h after fertilization. Aliquots of embryo suspension (5 mL) were transferred into each well followed by addition of the corresponding amount of compound solution to obtain the required final concentration. The concentration of the solvent did not exceed the maximal tolerated value (1% for ethanol and 0.05% for DMSO). In the series of trials, the concentration of compounds was sequentially decreased twofold until the effect disappeared. The activity of the compound was estimated as an effective threshold concentration, EC, resulting in cleavage alteration or developmental abnormalities. The embryo development was monitored using a Biolom LOMO light microscope (Saint-Petersburg).
Cytotoxicity in 60 human cancer cell lines was studied at the National Cancer Institute of USA according to the procedure described at http://dtp.nci.nih.gov/branches/btb/ivclsp.html.

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References

1. N.-H. Nam, *Curr. Med. Chem.*, 2003, 10, 1697.
2. G. R. Pettit, C. Jr. Temple, V. L. Narayanan, R. Varma, M. J. Simpson, M. R. Boyed, G. A. Rener, N. Bansal, *Anticancer Drug Des.*, 1995, 10, 299.
3. D. G. I. Kingston, *J. Nat. Prod.*, 2009, 72, 507.
4. K. Odlo, J. Hentzen, J. F. Chabert, S. Ducki, O. A. B. S. Gani, I. Sylte, M. Skrede, V. A. Florenes, T. V. Hansen, *Bioorg. Med. Chem.*, 2008, 16, 4829.
5. T. M. Beale, P. J. Bond, J. D. Brenton, D. S. Charnock-Jones, S. V. Ley, R. M. Myers, *Bioorg. Med. Chem.*, 2012, 20, 1749.
6. G. C. Tron, F. Pagliai, D. E. Grosso, A. A. Genazzani, G. Sorba, *J. Med. Chem.*, 2005, 48, 3260.
7. P. H. Olesen, A. R. Srensen, B. Urs, P. Kurtzhals, A. N. Bowler, U. Ehrbar, B. F. Hansen, *J. Med. Chem.*, 2003, 46, 3333.
8. (a) M. F. Schmidt, A. Isidro-Llobet, M. Lisurek, A. El Dahshan, J. Tan, R. Hilgenfeld, J. Rademann, *Angew. Chem., Int. Ed.*, 2008, 47, 3275; (b) Pat. RF 2158265, *Byul. Isobret.* [Invention Bull.], 2000, No. 30 (in Russian).
9. L. V. Batog, L. S. Konstantinova, V. Yu. Rozhkov, Yu. A. Strelenko, O. V. Lebedev, L. I. Khmel’’nitskii, *Khim. Geterotsikl. Soedin.*, 2000, 100 [Chem. Heterocycl. Compd. (Engl. Transl.)], 2000, 36, 91.
10. L. V. Batog, V. Yu. Rozhkov, M. I. Struchkova, *Mendelev Commun.*, 2002, 12, 159.
11. V. Yu. Rozhkov, L. V. Batog, E. K. Shevtsova, M. I. Struchkova, *Mendelev Commun.*, 2004, 14, 76.
12. V. Yu. Rozhkov, L. V. Batog, M. I. Struchkova, *Russ. Chem. Bull. (Int. Ed.)*, 2005, 54, 1866 [Izv. Akad. Nauk, Ser. Khim., 2005, 1923].
13. L. V. Batog, L. S. Konstantinova, V. Yu. Rozhkov, *Russ. Chem. Bull. (Int. Ed.)*, 2005, 54, 1915 [Izv. Akad. Nauk, Ser. Khim., 2005, 1859].
14. V. Yu. Rozhkov, L. V. Batog, M. I. Struchkova, *Mendelev Commun.*, 2008, 18, 161.
15. V. Yu. Rozhkov, L. V. Batog, M. I. Struchkova, *Russ. Chem. Bull. (Int. Ed.)*, 2011, 60, 1712 [Izv. Akad. Nauk, Ser. Khim., 2011, 1687].
16. R. S. Jacobs, L. Wilson, in *Modern Analysis of Antibiotics*, Ed. A. Aszalor, Marcel Dekker Inc., New York, 1986, p. 481.
17. N. Fusetani, in *Bioorganic Marine Chemistry*, Ed. P. J. Scheuer, Springer-Verlag, Berlin, 1987, Vol. 1, p. 61.
18. M. N. Semenova, A. S. Kiselyov, V. V. Semenov, *BioTechniques*, 2006, 40, 765.
19. M. N. Semenova, A. S. Kiselyov, D. V. Tsyganov, L. D. Konyushkin, S. I. Firgang, R. V. Semenov, O. R. Malyshev, M. M. Raihlstat, F. Fuchs, A. Stielow, M. Lantow, A. A. Philchenkov, M. P. Zavelevich, N. S. Zefirov, S. A. Kuznetsov, V. V. Semenov, *J. Med. Chem.*, 2011, 54, 7138.
20. V. V. Semenov, A. S. Kiselyov, I. Y. Titov, I. K. Sagamova, N. N. Ikizalp, N. B. Chernysheva, D. V. Tsyganov, L. D. Konyushkin, S. I. Firgang, R. V. Semenov, I. B. Karmanova, M. M. Raihlstat, M. N. Semenova, *J. Nat. Prod.*, 2010, 73, 1796.
21. A. Pelter, R. S. Ward, D. J. Watson, I. R. Jack, *J. Chem. Soc., Perkin Trans. 1*, 1982, 183.
22. Y. Zhu, X. Zou, F. Hu, C. Yao, B. Liu, H. Yang, *J. Agric. Food Chem.*, 2005, 53, 9566.
23. M. G. Ferklin, G. Chiarelotto, V. Gasparotto, L. D. Via, V. Pezzi, L. Barzon, G. Palu, I. Castagliuolo, *J. Med. Chem.*, 2005, 48, 3417.

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