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Modification of Sesquiterpene Lactones—Dehydrocostus Lactone and Alantolactone—by Click Chemistry Method. Cytotoxic Activity of the Obtained Conjugates

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Abstract—A method for modifying sesquiterpene lactones using the click chemistry methodology has been developed. A series of conjugates of alantolactone and dehydrocostus lactone with alkoxy substituted benzylazides was obtained and their cytotoxic profile with respect to tumor cells of the A549, SH-SY5Y, Hep-2 and HeLa lines was evaluated. It has been shown that derivatives containing dehydrocostus lactone motif in their structure exhibit the highest cytotoxic activity.

Keywords: sesquiterpene lactones, alantolactone, dehydrocostus lactone, click chemistry, cycloaddition, azides, acetylenes, cytotoxicity

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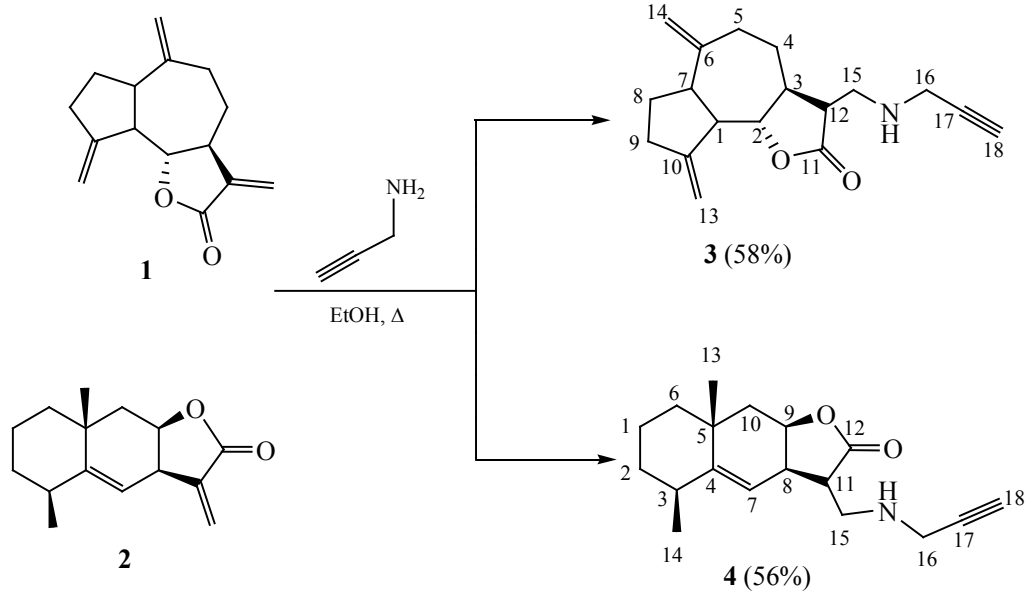
Products of natural origin are considered as valuable renewable raw materials for the development of promising therapeutic agents [1–6], and the creation of drugs from natural sources, especially from plants, has long been the basis for the treatment of various human diseases, including oncological pathologies. In recent years, there has been an increased interest in sesquiterpene lactones due to their antitumor effect on various tumor cell lines [7–9]. The biological effect of sesquiterpene lactones is realized by inducing apoptosis, as a result of their influence on the formation of reactive oxygen species, leading to oxidative damage in the cell and triggering the mitochondria-dependent pathway of apoptosis [9]. The cytotoxic effect, as a rule, is characteristic of lactones containing a conjugated double bond in the lactone ring, which facilitates nucleophilic addition reactions. Since various molecules with nucleophilic groups are present in a living cell, they are the targets for the action of sesquiterpene lactones. In this regard, various scientific groups are actively studying the ways of structural modification of such molecules and biological studies of various derivatives are being carried out.

Herein, we reported the synthesis of derivatives of sesquiterpene lactones, dehydrocostus lactone **1** and alantolactone **2**, and their cytotoxic activity towards various tumor cell lines. The choice of objects of the study is not accidental, since alantolactone **2** is one of the actively studied eudesmane-type sesquiterpene lactones with a wide spectrum of biological activity, including antitumor effects on neoplastic cells [10]. In addition, for dehydrocostus lactone **1** and some of its derivatives, a rather high cytostatic activity was also found [11, 12].

As the main methodology for the structural modification of the selected lactones, we chose an approach based on the use of the cycloaddition reaction of organic azides to acetylene derivatives. This technique of click chemistry has been widely used in recent years [13–15] due to the ease of implementation and almost quantitative yield of the target compounds. In addition, it should be noted that the triazole spacer formed as a result of click reactions is more than just a passive linker. It significantly facilitates

Scheme 1.



binding to biological targets due to hydrogen bonds, and also significantly increases solubility and dipole interactions [16].

Recently, a work has been published demonstrating the successful application of the click chemistry reaction to dehydrocostus lactone 1 [17]. The authors synthesized a series of 1,4-disubstituted-1,2,3-triazole conjugates based on dehydrocostus lactone with moderate cytotoxicity.

Since the click chemistry method involves the use of two synthetic blocks, acetylene and azide, we proposed to use the products of nucleophilic addition of propargylamine to lactones **1** and **2** as acetylene blocks. The reaction was carried out in boiling ethanol for 8 h (Scheme 1).

Both propargylated lactones **3** and **4**, which are a mixture of diastereoisomers isolated by column chromatography in 58 and 56% yields, respectively, are viscous oils, the structure of which was determined by a complex of spectral methods (^{1}H, ^{13}C NMR, IR spectroscopy and mass spectrometry), and composition by elemental analysis data.

The most strong arguments confirming the structure of compounds **3** and **4** are the presence of triplet signals in the ^{1}H NMR spectra in the region of 2.25 ppm with a characteristic spin-spin coupling constant $^4J_{HH}$ 2.4 Hz, as well as the presence in the ^{13}C NMR spectra of two signals of *sp*-hybridization carbon atoms in the 70–80 ppm region. Additional confirmation of the structure of compounds **3** and **4** are the data of IR spectroscopy: the presence of weak absorption bands in the 2100 cm^{-1} region.

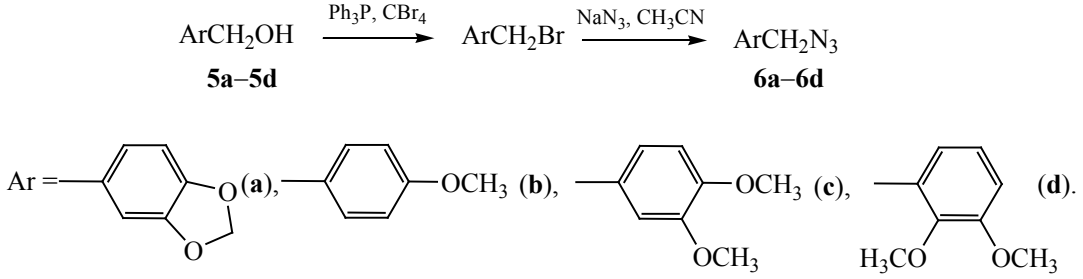
As the second azide block, it was proposed to use substituted benzyl azides **6a**–**6d**, which were obtained in two stages according to the known procedures [18–20] using benzyl alcohols **5a**–**5d** as starting compounds (Scheme 2). Azides **6a**–**6d** were isolated individually using column chromatography. The physico-chemical characteristics and spectral data for compounds **6a**–**6d** fully correspond to the literature data [18–20].

The reaction of acetylenes **3** and **4** with azides **6a**–**6d** was carried out under standard click chemistry conditions (Scheme 3). The reaction progress was monitored by thin layer chromatography.

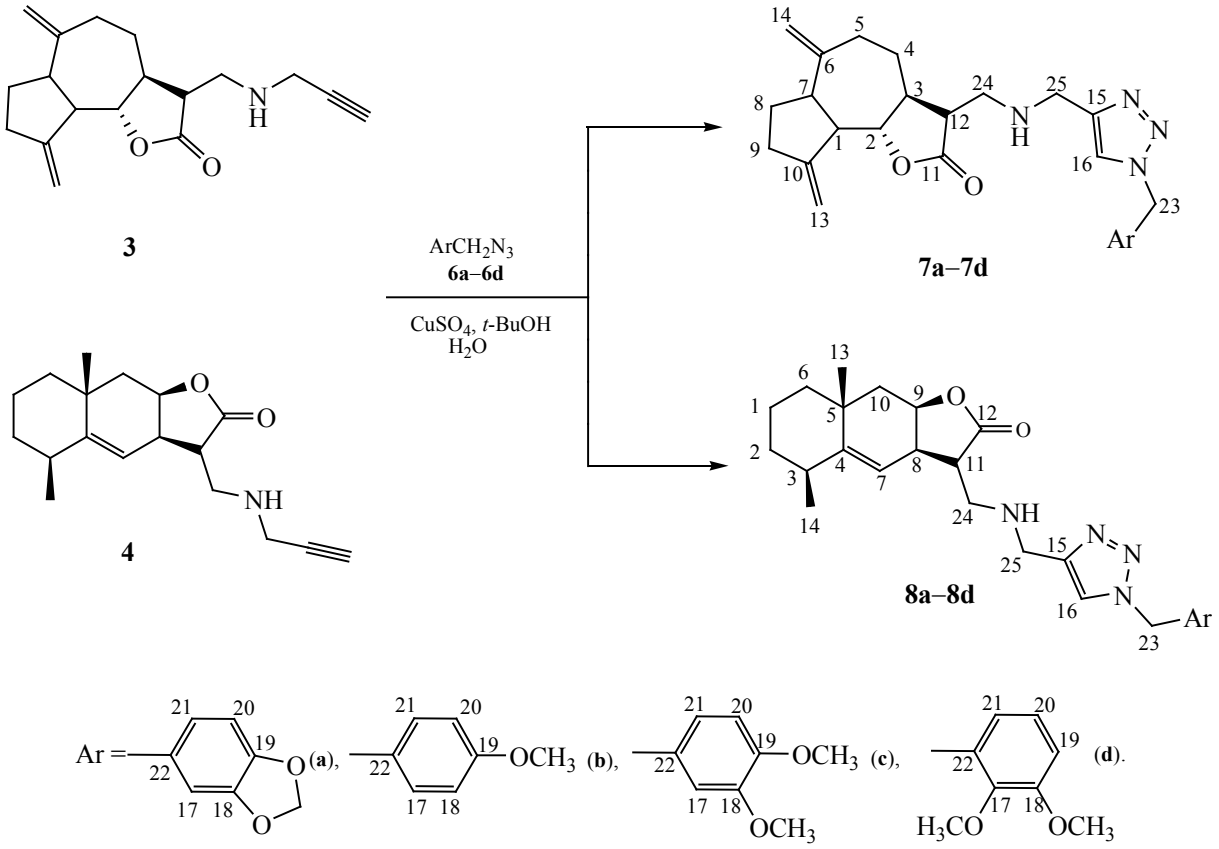
The target products, which are mixtures of diastereoisomers, were isolated by column chromatography on silica gel using a mixture of $CHCl_3$–EtOH (100:0.5→100:10) as an eluent. Their structure was proved by spectral methods (^{1}H and ^{13}C NMR, IR spectroscopy and mass spectrometry), and their composition was proved by analytical data.

The formation of the triazole ring is accompanied by the disappearance of signals from the C≡CH group in the spectra of the products and the appearance of new signals that unambiguously indicate the presence of such

Scheme 2.



Scheme 3.



a ring. For example, the ^{1}H NMR spectra of compounds **7** and **8** contain a characteristic downfield singlet signal at 7.39–7.54 ppm of the C^{16}H proton. The spectra of compounds **7**, **8** contain all signals related to the initial acetylenic and azide components, not affected by the formation of a new ring.

Since the main goal of studying the biological activity of the synthesized derivatives of sesquiterpene lactones was to study their antitumor activity, it was necessary to determine their cytotoxic profile, since one of the key properties of potential antineoplastic agents for the treatment of oncopathologies is the presence of cytotoxicity towards tumor cells. The effect of new derivatives of sesquiterpene lactones on the survival of cell cultures was determined by the IC_{50} value in relation to a number of tumor cells of the lines A549, SH-SY5Y,

Table 1. Cytotoxicity of sesquiterpene lactone derivatives towards tumor cell lines

Compound	IC_{50}, μM			
	Hep-2	Hela	A549	SH-SY5Y
7a	>100	18±0.32	>100	19.31±0.57
7b	26.28±0.38	16.60±0.59	25.20±3.44	16.14±0.46
7c	82.16±1.15	26.40±2.39	>100	21.83±0.02
7d	>100	79.59±0.33	>100	89.47±2.57
8a	>100	88.86±0.21	95.93±1.50	71.78±2.00
8b	>100	72.79±0.07	70.33±1.17	60.38±0.39
8c	>100	89.35±0.40	91.22±1.54	56.08±1.06
8d	>100	88.22±1.27	>100	>100

Hep-2 and HeLa. Viability was examined using the MTT test. As can be seen from Table 1, compound **7b** exhibits the most pronounced toxic effect with respect to all cell cultures, as evidenced by the IC_{50} values of the cytotoxic effect, which do not exceed 26 μM. In turn, compounds **7a** and **7c** exhibited similar activity on HeLa and SH-SY5Y cell lines, but without a pronounced effect on the viability of Hep-2 and A549 cells. For derivatives **8a**–**8c**, no cytotoxic effect was found on any of the cell lines in the selected concentration range. It should be noted that all compounds, except for derivative **7b**, had no effect on the Hep-2 line.

Summarizing the data in Table 1, we can conclude that derivatives **7a**–**7c** exhibit the highest cytotoxic activity, containing in their structure a fragment of dihydrocostus lactone, a natural sesquiterpene lactone obtained from various medicinal plants, such as *Inula helenium L.* and *Saussurea lappa* [21]. Studies by other authors have shown that dehydrocostus lactone has antitumor properties associated with reduced NF-kB activity [22], is able to induce both internal and external signaling pathways of apoptosis by activating caspase 9 [23] and 8, respectively, and also activates the effector caspase 3 [24], leading to DNA fragmentation.

In conclusion, using the click chemistry methodology, we obtained a number of previously undescribed conjugates of alantolactone and dehydrocostus lactone with aromatic alkoxy derivatives. It was found that dehydrocostus lactone derivatives showed the greatest cytotoxic effect towards A549, SH-SY5Y, Hep-2, and HeLa tumor cells.

EXPERIMENTAL

Chemically pure organic solvents were dehydrated and purified according to standard procedures. $CDCl_3$ solvent (Acros) was used without further purification.

^1H and $^{13}C\{^1H\}$ NMR spectra were recorded on a Bruker AV-400 instrument in $CDCl_3$ solutions using the residual proton signal of the deuterated solvent as an internal standard (^1H, ^{13}C). The ^{13}C NMR spectra were recorded in the JMODECHO mode; the signals of carbon atoms with an even and odd number of protons have opposite polarities. IR spectra were recorded on a Magna-IR 750 Nicolet IR Fourier spectrometer (thin layer). The reaction progress was monitored by TLC on Alumina TLC Plates w/UV254. Chromatographic purification of substances was carried out on Macherey-Nagel silica gel (MN Kieselgel 60, 70–230 mesh). Elemental analysis was carried out at the Microanalysis Laboratory of the A.N. Nesmeyanov Institute of Organoelement Compounds of RAS. Analysis by liquid chromatography–mass spectrometry (LC-MS) was performed on a Shimadzu LCMS-2020 instrument (Japan) using electrospray ionization (ESI). HPLC grade acetonitrile was used as the mobile phase.

Alantolactone and dehydrocostus lactone were isolated from plant substrates according to previously reported procedures [25, 26].

General procedure for the propargylation of dehydrocostus lactone 1 and alantolactone 2. The propargylation of lactones **1** and **2** was carried out in accordance with the previously described procedure [27]. A mixture of lactone **1** or **2** (0.5 mmol) and

propargylamine (55 mg, 1 mmol) in EtOH (5.0 mL) was refluxed for 8 h. After cooling, the solvent was removed in vacuum. The residue was purified using silica gel column chromatography, eluent—petroleum ether–AcOEt (3 : 1→1 : 1). After removal of the solvent, the residue was dried in vacuum (0.1 mmHg).

6,9-Dimethylene-3-[(prop-2-ylamino)methyl]-decahydroazuleno[4,5-*b*]furan-2(9b*H*)-one (3). Yield 82 mg (58%), diastereomers mixture, colorless oil. IR spectrum, ν, cm^{-1}: 650, 895, 999, 1112, 1129, 1176, 1207, 1320, 1337, 1442, 1460, 1641, 1766 (C=O), 2856, 2926, 3290. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 2.15–1.80 m and 1.37–1.25 m (6H, CH-terpene ring), 2.25 t (1H, $C^{18}H$, $^4J_{HH}$ 2.4 Hz), 2.60–2.40 m and 2.35–2.25 m (5H, CH-terpene ring), 3.05–2.80 m (4H, $C^{15}H_2$ + $C^{12}H$ + $C^{13}H$), 3.46 d (2H, $C^{16}H_2$, $^4J_{HH}$ 2.4 Hz), 3.97 t (1H, C^2H, $^3J_{HH}$ 9.2 Hz), 4.78 s (1H, $C^{14}H_B$), 4.88 s (1H, $C^{14}H_A$), 5.05 d (1H, $C^{13}H_B$, $^4J_{HH}$ 1.6 Hz), 5.18 d (1H, $C^{13}H_A$, $^4J_{HH}$ 1.6, $C^{13}H_A$). $^{13}C\{^1H\}$ NMR spectrum ($CDCl_3$,) δ_C, ppm: 30.00 (C^8H_2), 32.35 (C^4H_2), 32.38 (C^5H_2), 37.45 (C^9H_2), 38.41 ($C^{15}H_2$), 44.95 (C^3H), 46.42 ($C^{16}H_2$), 46.84 (C^7H), 47.19 (C^1H), 51.66 ($C^{12}H$), 71.69 ($C^{18}H$), 81.33 (C^{17}), 85.63 (C^2H), 109.00 ($C^{14}H_2$), 111.76 ($C^{13}H_2$), 149.64 (C^6), 151.51 (C^{10}), 177.45 (C^{11}=O). Mass spectrum (LC-MS ESI), *m*/*z* (I_{rel}, %): 286.000 (7.66) $[M + H]^+$, 593.150 (2.60) $[2M + Na]^+$. Found, %: C 75.51; H 8.13; N 4.73. $C_{18}H_{23}NO_2$. Calculated, %: C 75.76; H 8.12; N 4.91.

(5*S*,8a*R*)-5,8a-Dimethyl-3-(prop-2-ylamino)-methyl-3,3a,6,7,8,8a,9,9a-octahydronaphtho[2,3-*b*]-furan-2(5*H*)-one (4). Yield 80 mg (56%), diastereomers mixture, colorless oil. IR spectrum, ν, cm^{-1}: 668, 895, 947, 971, 1000, 1038, 1120, 1150, 1181, 1210, 1326, 1338, 1376, 1458, 1687, 1759 (C=O), 2868, 2928, 3289, 3307. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.22 s (3H, $C^{13}H_3$), 1.11 d (3H, $C^{14}H_3$, $^3J_{HH}$ 8.0 Hz), 2.12–2.04 м, 1.83–1.80 m and 1.61–1.40 m (9H, CH-terpene ring), 2.24 t (1H, $C^{18}H$, $^4J_{HH}$ 3.8 Hz), 3.14–2.98 m, 2.85–2.81 m and 2.49–2.46 m (5H, $C^{15}H_2$ + CH-terpene ring), 3.47 d (2H, $C^{16}H_2$, $^4J_{HH}$ 3.8 Hz), 4.76–4.74 m (1H, C^9H), 5.18 d (1H, C^7H, $^3J_{HH}$ 3.0 Hz). $^{13}C\{^1H\}$ NMR spectrum ($CDCl_3$,) δ_C, ppm: 16.62 (C^1H_2), 22.71 ($C^{13}H_3$), 28.45 ($C^{14}H_3$), 32.62 (C^2H_2), 32.79 (C^6H_2), 37.43 ($C^{10}H_2$), 38.18 (C^8H), 38.24 (C^3H), 41.96 (C^5), 42.50 ($C^{15}H_2$), 45.28 ($C^{16}H_2$), 45.46 ($C^{11}H$), 71.63 ($C^{18}H$), 77.20 (C^9H), 81.37 (C^{17}), 114.81 (C^7H), 150.87 (C^4), 177.56 (C^{12}=O). Mass spectrum (LC-MS ESI), *m*/*z* (I_{rel}, %): 288.050 (7.75) $[M + H]^+$. Found, %: C 67.90; H 7.01; N 4.42. $2C_{18}H_{25}NO_2 \cdot CH_2Cl_2$. Calculated, %: C 67.36; H 7.94; N 4.25.

General procedure for the synthesis of triazoles 7a–8d and 8a–8d. A mixture of acetylene **3** or **4** (0.5 mmol), *t*-BuOH (5 mL), H_2O (2 mL), $CuSO_4 \cdot 5H_2O$ (5 mol%) was stirred for 10 min, then sodium ascorbate (~20 mg) and the corresponding azide **6** (0.5 mmol) was added with stirring. The resulting mixture was stirred for 24 h. After completion of the reaction, the solvent was removed in vacuum and DCM (10 mL) was added to the residue. The organic layer was separated, DCM was removed in vacuum, the residue was chromatographed on silica gel (eluent—$CHCl_3$–EtOH, 100 : 0.5→100 : 10). After removing the solvent, the residue was kept in vacuum (0.1 mmHg) for 5 h.

3-({[1-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-1*H*-1,2,3-triazol-4-yl]methylamino}methyl)-6,9-dimethylenene-decahydroazuleno[4,5-*b*]furan-2(9b*H*)-one (7a). Yield 81%, diastereomers mixture, yellow oil. IR spectrum, ν, cm^{-1}: 515, 683, 775, 896, 927, 997, 1038, 1177, 1210, 1249, 1336, 1337, 1447, 1492, 1504, 1641, 1765 (C=O), 2857, 3080. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.25–1.37 m, 1.80–2.10 m, 2.19–2.28 m, 2.40–2.55 m, 2.79–2.97 m (14H, CH-terpene ring + $C^{24}H_2$), 3.92 s (2H, $C^{25}H_2$), 3.95 t (1H, C^2H, $^3J_{HH}$ 8.0), 4.76 br. s, 4.86 br. s, 5.03 br. s and 5.16 br. s (4H, $C^{14}H_2$ + $C^{13}H_2$), 5.40 s (2H, $C^{23}H_2$), 5.96 s (2H, OCH_2O), 6.75 s (1H, $C^{17}H$), 6.79 br. s (2H, $C^{21}H$ + $C^{20}H$), 7.45 s (1H, $C^{16}H$). $^{13}C\{^1H\}$ NMR spectrum ($CDCl_3$,) δ_C, ppm: 29.98 (C^4H_2), 32.37 (C^9H_2 + C^8H_2), 37.40 (C^5H_2), 44.64 ($C^{12}H$), 44.93 ($C^{25}H_2$), 46.80 (C^1H), 46.83 ($C^{24}H_2$), 47.00 (C^7H), 51.64 (C^3H), 53.80 ($C^{23}H_2$), 85.66 (C^2H), 101.23 (OCH_2O), 108.41 ($C^{17}H$), 108.93 ($C^{14}H_2$), 111.73 ($C^{13}H_2$), 121.39 ($C^{21}H$), 121.81 ($C^{20}H$), 128.09 (C^{22}), 146.52 (C^{15}), 147.85 (C^6), 148.10 (C^{10}), 149.60 (C^{19}), 151.53 (C^{18}), 177.63 (C^{11}=O). Mass spectrum (LC-MS ESI), *m*/*z* (I_{rel}, %): 463.050 (10.28) $[M + H]^+$, 485.100 (79.00) $[M + Na]^+$. Found, %: C 61.07; H 5.81; N 10.27. $1.25C_{26}H_{30}N_4O_4 \cdot CH_2Cl_2$. Calculated, %: C 60.68; H 6.00; N 10.56.

3-({[1-(4-Methoxybenzyl)-1*H*-1,2,3-triazol-4-yl]-methylamino}methyl)-6,9-dimethylenedecahydro-azuleno[4,5-*b*]furan-2(9b*H*)-one (7b). Yield 78%, diastereomers mixture, yellow oil. IR spectrum, ν, cm^{-1}: 514, 666, 755, 895, 998, 1033, 1125, 1178, 1210, 1250, 1335, 1441, 1462, 1515, 1613, 1641, 1766 (C=O), 2855, 2932, 3080. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.24–1.34 m, 1.82–2.05 m, 2.17–2.24 m, 2.34–2.51 m, 2.75–2.94 m (14H, CH-terpene ring + $C^{24}H_2$), 3.78 s and 3.88 s (5H, $C^{25}H_2$ + OCH_3), 3.92 t (1H, C^2H, $^3J_{HH}$ 8.0 Hz), 4.74 br. s, 4.84 br. s, 5.01 br. s, 5.15 br. s (4H,

$C^{14}H_2$ + $C^{13}H_2$), 5.42 s (2H, $C^{23}H_2$), 6.87 d (2H, $C^{17}H$ + $C^{21}H$, $^3J_{HH}$ 8.0 Hz), 7.21 d (2H, $C^{18}H$ + $C^{20}H$, $^3J_{HH}$ 8.0 Hz), 7.39 s (1H, $C^{16}H$). $^{13}C\{^1H\}$ NMR spectrum ($CDCl_3$) δ_C, ppm: 29.91 (C^4H_2), 32.38 (C^9H_2 + C^8H_2), 37.37 (C^5H_2), 44.66 ($C^{25}H_2$), 44.80 (C^1H), 46.72 ($C^{12}H$), 46.79 ($C^{24}H_2$), 47.05 (C^7H), 51.58 (C^3H), 53.40 ($C^{23}H_2$), 55.08 (OCH_3), 85.54 (C^2H), 108.83 ($C^{14}H_2$), 111.63 ($C^{13}H_2$), 114.18 ($C^{17}H$ + $C^{21}H$), 121.17 ($C^{16}H$), 126.43 (C^{22}), 129.44 ($C^{18}H$ + $C^{20}H$), 146.63 (C^{15}), 149.55 (C^6), 151.50 (C^{10}), 159.61 (C^{19}), 177.56 (C^{11}=O). Mass spectrum (LC-MS ESI), *m*/*z* (I_{rel}, %): 449.150 (11.82) $[M + H]^+$, 471.050 (72.46) $[M + Na]^+$, 919.250 (100.00) $[2M + Na]^+$. Found, %: C 64.46; H 6.51; N 11.29. $1.75C_{26}H_{32}N_4O_3 \cdot CH_2Cl_2$. Calculated, %: C 64.20; H 6.72; N 11.27.

3-[{(1-(3,4-Dimethoxybenzyl)-1*H*-1,2,3-triazol-4-yl]methylamino}methyl)-6,9-dimethylenedecahydroazuleno[4,5-*b*]furan-2(9b*H*)-one (7c). Yield 80%, diastereomers mixture, yellow oil. IR spectrum, ν, cm^{-1}: 555, 666, 753, 894, 998, 1026, 1048, 1142, 1160, 1240, 1263, 1336, 1441, 1464, 1517, 1594, 1641, 1764 (C=O), 2853, 2931, 3080. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.21–1.37 m, 1.80–2.05 m, 2.19–2.27 m, 2.38–2.55 m, 2.76–2.96 m (14H, CH-terpene ring + $C^{24}H_2$), 3.85 s and 3.88 s (6H, OCH_3), 3.93 s (2H, $C^{25}H_2$), 3.95 t (1H, C^2H $^3J_{HH}$ 8.0 Hz), 4.77 br. s, 4.87 br. s, 5.03 br. s, 5.16 br. s (4H, $C^{14}H_2$ + $C^{13}H_2$), 5.44 s (2H, $C^{23}H_2$), 6.81–6.88 m (3H, $C^{21}H$ + $C^{20}H$ + $C^{17}H$), 7.45 s (1H, $C^{16}H$). $^{13}C\{^1H\}$ NMR spectrum ($CDCl_3$,) δ_C, ppm: 30.00 (C^4H_2), 32.36 (C^9H_2 + C^8H_2), 37.38 (C^5H_2), 44.67 ($C^{25}H_2$), 44.98 (C^3H), 46.87 ($C^{24}H_2$), 47.02 (C^1H), 51.68 (C^7H), 53.92 ($C^{23}H_2$), 55.82 and 55.79 (OCH_3), 85.66 (C^2H), 108.98 ($C^{14}H_2$), 111.08 ($C^{20}H$), 111.12 ($C^{21}H$), 111.79 ($C^{13}H_2$), 120.74 ($C^{17}H$), 121.42 ($C^{16}H$), 126.85 (C^{22}), 146.50 (C^6), 149.27(C^{10}), 149.58 (C^{19}), 151.52 (C^{18}), 177.64 (C^{11}=O). Mass spectrum (LC-MS ESI), *m*/*z* (I_{rel}, %): 479.150 (13.64) $[M + H]^+$, 501.100 (68.57) $[M + Na]^+$, 979.300 (100.00) $[2M + Na]^+$. Found, %: C 61.67; H 6.66; N 10.02. $1.25C_{27}H_{34}N_4O_4 \cdot CH_2Cl_2$. Calculated, %: C 61.09; H 6.57; N 10.25.

3-({[1-(2,3-Dimethoxybenzyl)-1*H*-1,2,3-triazol-4-yl]methylamino}methyl)-6,9-dimethylenedecahydroazuleno[4,5-*b*]furan-2(9b*H*)-one (7d). Yield 82%, diastereomers mixture, yellow oil. IR spectrum, ν, cm^{-1}: 502, 667, 754, 894, 1002, 1048, 1076, 1176, 1227, 1272, 1433, 1485, 1589, 1641, 1699, 1766 (C=O), 2854, 2934, 3081. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.27–1.33 m, 1.92–2.05 m, 2.44–2.52 m, 2.80–2.95 m (14H, CH-terpene ring + $C^{24}H_2$), 3.84 s and 3.88 s (6H, OCH_3), 3.93 s (2H, $C^{25}H_2$), 3.95 t (1H, C^2H, $^3J_{HH}$ 8.0 Hz), 4.77 br. s, 4.87 br. s, 5.05 br. s, 5.18 br. s (4H, $C^{14}H_2$ + $C^{13}H_2$), 5.54 br. s (3H, $C^{23}H_2$ + NH), 6.83 d (1H, $C^{19}H$, $^3J_{HH}$ 8.0 Hz), 6.93 d (1H, $C^{21}H$, $^3J_{HH}$ 8.0 Hz), 7.04 t (1H, $C^{20}H$ $^3J_{HH}$ 8.0 Hz), 7.54 s (1H, $C^{16}H$). $^{13}C\{^1H\}$ NMR spectrum ($CDCl_3$,) δ_C, ppm: 30.00 (C^4H_2), 31.43 (C^8H_2), 32.37 (C^9H_2), 37.39 (C^5H_2), 44.36 ($C^{25}H_2$), 45.09 ($C^{12}H$), 46.66 ($C^{24}H_2$), 46.83 (C^7H), 48.86 ($C^{23}H_2$), 51.66 (C^3H), 55.66 and 60.75 (2 OCH_3); 85.82 (C^2H), 108.99 ($C^{14}H_2$), 111.80 ($C^{13}H_2$), 113.02 ($C^{19}H$), 121.51 ($C^{20}H$), 122.48 ($C^{16}H$), 124.32 ($C^{21}H$), 128.14 (C^{22}), 145.07 (C^{15}), 146.86 (C^6), 149.59 (C^{10}), 151.52 (C^{17}), 151.73 (C^{18}), 177.58 (C^{11}=O). Mass spectrum (LC-MS ESI), *m*/*z* (I_{rel}, %): 479.100 (13.38) $[M + H]^+$, 501.100 (92.53) $[M + Na]^+$, 979.300 (100.00) $[2M + Na]^+$. Found, %: s 57.37; H 6.24; N 9.02. $C_{27}H_{34}N_4O_4 \cdot 1.4 \cdot CH_2Cl_2$. Calculated, %: s 57.09; H 6.21; N 9.38.

(5*S*,8a*R*)-3-{[(1-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-1*H*-1,2,3-triazol-4-yl]methylamino}methyl)-5,8a-dimethyl-3 ,3a,6,7,8,8a,9,9a-octahydronaphtho[2,3-*b*]-furan-2(5*H*)-one (8a). Yield 78%, diastereomers mixture, yellow oil. IR spectrum, ν, cm^{-1}: 518, 669, 775, 811, 927, 1120, 1039, 1149, 1181, 1216, 1249, 1339, 1376, 1447, 1492, 1504, 1758 (C=O), 2929, 3139. ^1H NMR spectrum ($CDCl_3$), δ, ppm : 1.08 d (3H, $C^{14}H_3$ $^3J_{HH}$ 8.0 Hz), 1.12 s (3H, $C^{13}H_3$), 1.07–1.12 m, 1.39–159 m, 1.74–1.85 m, 2.05–2.10 m, 2.35–2.50 m, 2.75–2.85 m, 2.95–3.05 m, 3.09–3.11 m (14H, $C^{24}H_2$ + CH-terpene ring), 3.98 d (1H, $C^{25}H_B$ $^2J_{HH}$ 12.0 Hz), 3.94 d (1H, $C^{25}H_A$, $^2J_{HH}$ 12.0 Hz), 4.71–4.73 m (1H, C^9H), 5.10 d (1H, C^7H, $^3J_{HH}$ 4.0 Hz), 5.39 s (2H, $C^{23}H_2$), 5.96 s (2H, OCH_2O), 6.74 s (1H, $C^{20}H$), 6.78 br. s (2H, $C^{17}H$ + $C^{21}H$), 7.42 s (1H, $C^{16}H$). $^{13}C\{^1H\}$ NMR spectrum ($CDCl_3$,) δ_C, ppm: 16.62 (C^1H_2), 22.72 ($C^{14}H_3$), 28.45 ($C^{13}H_3$), 32.61 (C^2H_2), 32.78 (C^6H_2), 37.41 (C^8H), 38.17 (C^3H), 41.97 ($C^{25}H_2$), 42.47 ($C^{10}H_2$), 44.59 ($C^{24}H_2$), 45.49 ($C^{11}H$), 45.73 ($C^{23}H_2$), 53.76 (C^5), 77.21 (C^9H), 101.20 (OCH_2O), 108.37 ($C^{21}H$), 108.41 ($C^{20}H$), 114.73 (C^7H), 121.27 ($C^{16}H$), 121.80 ($C^{17}H$), 128.01 (C^{22}), 147.82 (C^{15}), 148.07 (C^4), 146.56 and 150.87 (C^{18} + C^{19}), 177.67 (C^{12}=O). Mass spectrum (LC-MS ESI), *m*/*z* (I_{rel}, %): 465.100 (15.06) $[M + H]^+$, 487.100 (100.00) $[M + Na]^+$, 951.250 (96.51) $[2M + Na]^+$. Found, %: C 60.78; H 6.38; N 10.76. $1.3C_{26}H_{32}N_4O_4 \cdot CH_2Cl_2$. Calculated, %: C 60.82; H 6.39; N 10.61.

(5*S*,8a*R*)-3-({[1-(4-Methoxybenzyl)-1*H*-1,2,3-triazol-4-yl]methylamino}methyl)-5,8a-dimethyl-

3,3a,6,7,8,8a, 9,9a-octahydronaphtho[2,3-*b*]furan-2(5*H*)-one (8b). Yield 80%, diastereomers mixture, yellow oil. IR spectrum, ν, cm^{-1}:516, 554, 636, 669, 703, 786, 824, 896, 972, 1000, 1036, 1126, 1149, 1179, 1213, 1250, 1326, 1376, 1442, 1464, 1515, 1586, 1613, 1649, 1760 (C=O), 2868, 2929, 3137. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.07 d (3H, $C^{14}H_3$ $^3J_{HH}$ 8.0 Hz), 1.08–1.12 m (1H, CH-terpene ring), 1.20 s (3H, $C^{13}H_3$), 1.35–1.85 m, 2.05–2.20 m, 2.40–2.45 m, 2.77–3.15 m (10H, CH-terpene ring), 3.79 s (3H, OCH_3), 3.90 br. s (1H, NH), 3.92 d. t (1H, C^9H, $^3J_{HH}$ 12.0, C^9H), 4.73–5.10 br. s (2H, $C^{25}H_A + C^{25}H_B$), 5.43 br. s (2H, $C^{23}H_2$), 6.88 d (2H, $C^{17}H + C^{21}H$, $^3J_{HH}$ 8.0 Hz), 7.22 d (2H, $C^{18}H + C^{20}H$, $^3J_{HH}$ 8.0 Hz), 7.40 s (1H, $C^{16}H$). $^{13}C\{^1H\}$ NMR spectrum ($CDCl_3$,) δ_C, ppm: 16.22 (C^1H_2), 22.73 ($C^{14}H_3$), 28.45 ($C^{13}H_3$), 32.62 (C^2H_2), 32.79 (C^6H_2), 37.40 (C^8H), 38.17 (C^3H), 41.97 ($C^{25}H_2$), 42.47 ($C^{10}H_2$), 44.56 ($C^{24}H_2$), 45.45 ($C^{11}H$), 45.68 ($C^{23}H_2$), 53.47 (C^5), 55.12 (OCH_3), 77.22 (C^9H), 114.24 ($C^{17}H + C^{21}H$), 114.71 (C^7H), 121.30 ($C^{16}H$), 126.39 (C^{22}), 129.50 ($C^{18}H + C^{20}H$), 146.35 (C^{15}), 150.88 (C^4), 159.68 (C^{19}), 177.65 (C^{12}=O). Mass spectrum (LC-MS ESI), *m*/*z* (I_{rel}, %): 451.150 (14.29) $[M + H]^+$, 473.100 (93.93) $[M + Na]^+$, 923.350 (100.00) $[2M + Na]^+$. Found, %: C 63.64; H 6.59; N 10.91. $1.5C_{26}H_{34}N_4O_3 \cdot CH_2Cl_2$. Calculated, %: C 63.15; H 7.02; N 11.05.

(5*S*,8a*R*)-3-({[1-(3,4-Dimethoxybenzyl)-1*H*-1,2,3-triazol-4-yl]methylamino}methyl)-5,8a-dimethyl-3,3a,6,7,8, 8a,9,9a-octahydronaphtho[2,3-*b*]furan-2(5*H*)-one (8c). Yield 82%, diastereomers mixture, yellow. IR spectrum, ν, cm^{-1}: 556, 636, 667, 755, 860, 877, 970, 1027, 1143, 1160, 1180, 1241, 1264, 1325, 1376, 1422, 1442, 1465, 1518, 1594, 1608, 1758 (C=O), 2868, 2930, 3138. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.07 d (3H, $C^{14}H_3$, $^3J_{HH}$ 8.0 Hz), 1.09–1.13 m (1H, CH-terpene ring), 1.20 s (3H, $C^{13}H_3$), 1.40–1.85 m, 2.06–2.11 m, 2.35–2.50 m, 2.75–3.15 m (10H, CH-terpene ring), 3.84 s and 3.87 s (6H, OCH_3), 3.93 d. t (1H, C^9H, $^3J_{HH}$ 12.0 Hz), 4.74 br. s and 5.11 br. s (2H, $C^{25}H_A + C^{25}H_B$), 5.43 br. s (2H, $C^{23}H_2$), 6.80–6.90 m (2H, $C^{21}H + C^{17}H$), 7.43 s (1H, $C^{16}H$). $^{13}C\{^1H\}$ NMR spectrum ($CDCl_3$,) δ_C, ppm: 16.63 (C^1H_2), 22.74 ($C^{14}H_3$), 28.47 ($C^{13}H_3$), 32.64 (C^2H_2), 32.81 (C^6H_2), 37.45 (C^8H), 38.21 (C^3H), 41.99 ($C^{25}H_2$), 42.49 ($C^{10}H_2$), 44.58 ($C^{24}H_2$), 45.44 ($C^{11}H$), 45.72 ($C^{23}H_2$), 53.88 (C^5), 55.76 and 55.78 (2 OCH_3), 77.27 (C^9H), 111.09 (C^7H), 114.69 ($C^{20}H$), 120.72 ($C^{21}H$), 121.40 ($C^{16}H$), 126.75 (C^{22}), 146.37 (C^4), 149.24 (C^{19}), 150.98 (C^{18}), 177.72 (C^{12}=O). Mass spectrum (LC-MS ESI), *m*/*z* (I_{rel}, %): 481.200 (13.80) $[M + H]^+$, 503.150 (87.79) $[M + Na]^+$, 983.350 (100.00) $[2M + Na]^+$. Found, %: C 60.28; H 7.09; N 9.69. $1.25C_{27}H_{36}N_4O_4 \cdot CH_2Cl_2$. Calculated, %: C 60.87; H 6.91; N 10.21.

(5*S*,8a*R*)-3-({[1-(2,3-Dimethoxybenzyl)-1*H*-1,2,3-triazol-4-yl]methylamino}methyl)-5,8a-dimethyl-3,3a,6,7,8, 8a,9,9a-octahydronaphtho[2,3-*b*]furan-2(5*H*)-one (8d). Yield 85%, diastereomers mixture, yellow oil. IR spectrum, ν, cm^{-1}: 636, 337, 754, 896, 1003, 1048, 1077, 1125, 1149, 1178, 1228, 1273, 1325, 1377, 1432, 1465, 1486, 1589, 1695, 1760 (C=O), 2868, 2930, 3142. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: 1.08–1.7 m, 2.05–2.12 m, 2.35–2.46 m (18H, CH-terpene ring), 2.82–3.13 m (3H, $C^8H + C^{24}H_2$), 3.85 s, 3.88 s (6H, OCH_3), 4.75 br. s and 5.13 br. s (2H, $C^{25}H_A + C^{25}H_B$), 5.54 br. s (3H, $C^7H + C^{23}H_2$), 6.83 d (1H, $C^{21}H$, $^3J_{HH}$ 8.0 Hz), 6.93 d (1H, $C^{19}H$, $^3J_{HH}$ 8.0 Hz), 7.05 t (1H, $C^{20}H$, $^3J_{HH}$ 8.0 Hz), 7.54 s (1H, $C^{16}H$). $^{13}C\{^1H\}$ NMR spectrum ($CDCl_3$,) δ_C, ppm: 14.40 ($C^{14}H_3$), 16.69 (C^1H_2), 22.79 ($C^{13}H_3$), 28.51 (C^3H), 32.70 ($C^{25}H_2$), 32.85 ($C^{10}H_2$), 37.46 (C^8H), 38.23 ($C^{11}H$), 42.06 ($C^{24}H_2$), 42.56 ($C^{23}H_2$), 48.85 (C^5), 55.66.74 and 60.74 (2 OCH_3), 77.29 (C^9H), 113.06 ($C^{19}H$), 114.75 (C^7H), 121.46 ($C^{16}H$), 121.56 ($C^{20}H$), 124.31 ($C^{21}H$), 128.15 (C^{22}), 146.95 (C^4), 150.99 (C^{17}), 152.60 (C^{18}), 177.62 (C^{12}=O). Mass spectrum LC-MS (ESI), *m*/*z* (I_{rel}, %): 481.100 (37.73) $[M + H]^+$, 503.100 (100.00) $[M + Na]^+$, 983.250 (81.94) $[2M + Na]^+$. Found, %: C 55.36; H 6.18; N 9.39. $C_{27}H_{36}N_4O_4 \cdot 1.6CH_2Cl_2$. Calculated, %: C 55.72; H 6.41; N 9.09.

Study of the cytotoxic profile of sesquiterpene lactone derivatives. *Cell cultivation*. Cell cultures of tumor lines A549 (pulmonary adenocarcinoma), SH-SY5Y (neuroblastoma), Hep-2 (epidermoid carcinoma of the larynx), HeLa (cervical carcinoma) were provided by the Laboratory of Tumor Cell Genetics of the N.N. Blokhin National Medical Research Center of Oncology and Institute of Cytology of RAS. Cells were grown in DMEM (Gibco, UK) containing fetal bovine serum (10% v/v) (ThermoFisher Scientific, UK), L-glutamine (2 mM) (Gibco, UK) and penicillin-streptomycin (1% v/v) (PanEco, Russia) at 37°C in a humidified CO_2 atmosphere (5%). Upon reaching 80% confluence, the cells were passaged using a 0.25% trypsin-EDTA solution (PanEco, Russia).

Cell viability assay. Cell viability was determined using the MTT assay [27]. The method is based on the reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] by mitochondrial

dehydrogenases of living cells to crystals of insoluble formazan, thus, the amount of formazan formed reflects cell viability. Each cell line was seeded in 96-well plates (1×10^4 cells/well) and cultured for 24 h at 37°C under CO_2 (5%) atmosphere. Incubation with test substances at selected concentrations (0.1, 1, 10, 30, 100 μM) was carried out for 24 h, then MTT solution (5 mg/mL in 0.9% NaCl) was added to each well, cells were incubated for 2 h at 37°C. After removal of the nutrient medium, dimethyl sulfoxide was added to each well to dissolve the formazan. Using a Victor 3 plate analyzer (PerkinElmer), the absorbance at 530 nm was determined minus the measured background absorbance at 620 nm. The concentration value causing 50% inhibition of cell population growth (IC_{50}) was determined from dose-response curves using GraphPad Prism 9.0 software.

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CONFLICT OF INTERESTS

V.K. Brel is a member of the Editorial Board of the Russian Journal of General Chemistry. The other authors declare no conflicts of interest.

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REFERENCES

1. Du, L., Robles, A.J., King, J.B., Powell, D.R., Miller, A.N., Mooberry, S.L., and Cichewicz, R.H., *Angew. Chem. Int. Ed*., 2014, vol. 53, p. 804. https://doi.org/10.1002/anie.201306549
2. Bauer, A. and Brönstrup, M., *Nat. Prod. Rep*., 2014, vol. 31, p. 35. https://doi.org/10.1039/c3np70058e
3. Brown, D.G., Lister, T., and May-Dracka, T.L., *Bioorg. Med. Chem. Lett*., 2014, vol. 24. P.413. https://doi.org/10.1016/j.bmcl.2013.12.059
4. Scheepstra, M., Nieto, L., Hirsch, A.K.H., Fuchs, S., Leysen, S., Lam, C.V., Panhuis, L., Boeckel, C.A.A., Wienk, H., Boelens, R., Ottmann, C., Milroy, L.-G., and Brunsveld, L., *Angew. Chem. Int. Ed*., 2014, vol. 53, p. 6443. https://doi.org/10.1002/anie.201403773
5. Hattum, H. and Waldmann, H., *Am. Chem. Soc*., 2014, vol. 136, p. 11853. https://doi.org/10.1021/ja505861d
6. Harvey, A.L., Edrada-Ebel, R., and Quinn, R.J., *Nature Rev. Drug Discov*., 2015, vol. 14, p. 111. https://doi.org/10.1038/nrd4510
7. Xu, Y.-Z., Gu, X.-Y., Peng, S.-J., Fang, J.-G., Zhang, Y.-M., Huang, D.-J., Chen, J.-J., and Gao, K., *Eur. J. Med. Chem*., 2015, vol. 94, p. 284. https://doi.org/10.1016/j.ejmech.2015.03.001

8. Babaei, G., Aliarab, A., Abroon, S., Rasmi, Y., and Aziz, S.G.-G., *Biomed. Pharmacother*., 2018, vol. 106, p. 239. https://doi.org/10.1016/j.biopha.2018.06.131
9. Zhang, S., Won, Y.-K., Ong, C.-N., and Shen, H.M., *Curr. Med. Chem. Anti-Cancer Agents*, 2005, vol. 5, p. 239. https://doi.org/10.2174/1568011053765976
10. Rasul, A., Khan, M., Ali, M., Li, J., and Li, X., *Hindawi Publ. Corp. Sci. World J*., 2013, Article ID 248532. https://doi.org/10.1155/2013/248532
11. Kretschmer, N., Rinner, B., Stuendl, N., Kaltenegger, H., Wolf, E., Kunert, O., Boechzelt, H., Leithner, A., Bauer, R., and Lohberger, B., *Planta Med*., 2012, vol. 78, p. 1749. https://doi.org/10.1055/s-0032-1315385
12. Sun, C.-M., Syu, W.-J., Don, M.-J., Lu, J.-J., and Lee, G.-H., *J. Nat. Prod*., 2003, vol. 66, p. 1175. https://doi.org/10.1021/np030147e
13. Kolb, H.C., Finn, M.G., and Sharpless, K.B., *Angew. Chem. Int. Ed*., 2001, vol. 40, p. 2004. https://doi.org/10.1002/1521-3773(20010601)40:11<2004::AID-ANIE2004>3.0.CO;2-5
14. Artyushin, O.I., Sharova, E.V., Vinogradova, N.M., Genkina, G..K., Moiseeva, A.A., Klemenkova, Z.V., Orshanskaya, I.R., Shtro, A.A., Kadyrova, R.A., Zrubaev, V.V., Yarovaya, O.I., Salakhutdinov, N.F., and Brel, V.K., *Biorg. Med. Chem. Lett*., 2017, vol. 27, no. 10, p. 2181. https://doi.org/10.1016/j.bmcl.2017.03.051
15. Artyushin, O.I., Moiseeva, A.A., Zarubaev, V.V., Slita, A.V., Galochkina, A.V., Muryleva, A.A., Borisevich, S.S., Yarovaya, O.I., Salakhutdinov, N.F., and Brel, V.K., *Chem. Biodiversity*, 2019, vol. 16. e1900340. https://doi.org/10.1002/cbdv.201900340
16. Zhang, Q., Lu, Y., Ding, Y., Zhai, J., Ji, Q., Ma, Q., Yang, M., Fan, H., Long, J., Tong, Z., Shi, Y., Jia, Y., and Han, B., *J. Med. Chem*., 2012, vol. 55, p. 8757.
17. Kumar, Ch.P., Devi, A., Yadav, P.A., Vadaparthi, R.R., Shankaraiah, G., Sowjanya, P., Jain, N., and Babu, K.S., *J. Asian Nat. Prod. Res*., 2016, vol. 18, no. 11, p. 1063. https://doi.org/10.1080/10286020.2016.1193012
18. Schaefer, M., Hanik, N., and Kilbinger, A.F.M., *Macromolecules*, 2012, vol. 45, no. 17, p. 6807. https://doi.org/10.1021/MA301061Z
19. Maddani, M. and Prabhu, K.R., *Tetrahedron Lett*., 2008, vol. 49, no. 29–30, p. 4526. https://doi.org/10.1016/J.TETLET.2008.05.047
20. Lakshman, M.K., Singh, M.K., Kumar, M., Chamala, R.R., Yedulla, V.R., Wagner, D., Leung, E., Yang, L., Matin, A., and Ahmad, S., *Beilstein J. Org. Chem*., 2014, vol. 10, p. 1919. https://doi.org/10.3762/bjoc.10.200
21. Lin, X., Peng, Z., and Su, C., *Int. J. Mol. Sci*., 2015, vol. 16, p. 10888. https://doi.org/10.3390/ijms160510888
22. Nie, Y., Wang, Z., Chai, G., Xiong, Y., Li, B., Zhang, H., Xin, R., Qian, X., Tang, Z., Wu, J., and Zhao, P., *Molecules*, 2019, vol. 24, no. 8, p. 1510. https://doi.org/10.3390/molecules24081510
23. Woo, J.-H., Ahn, J.-H., Jang, D.S., and Choi, J.-H., *Am. J. Chin. Med*., 2019, vol. 47. N 6, p. 1289. https://doi.org/10.1142/S0192415X19500666
24. Li, Q., Wang, Z., Xie, Y., and Hu, H., *Biomed. Pharmacother*., 2020, vol. 125, p. 109955. https://doi.org/10.1016/j.biopha.2020.109955
25. Semakov, A. and Klochkov, S., *Khim Rast. Syr'ya*, 2020, 3, p. 145. https://doi.org/10.14258/jcprm.2020034681
26. Semakov, A.V., Anikina, L.V., and Klochkov, S.G., *Russ J Bioorg Chem*., 2021, vol. 47, p. 906. https://doi.org/10.1134/S106816202104018X
27. Matsuda, H., Kageura, T., Inoue, Y., Morikawa, T., and Yoshikawa, M., *Tetrahedron*, 2000, vol. 56, no. 39, p. 7763. https://doi.org/10.1016/S0040-4020(00)00696-7
28. Präbst, K., Engelhardt, H., Ringgeler, S., and Hübner, H., in *Cell Viability Assays. Methods in Molecular Biology*, Gilbert, D.F. and Friedrich, O. , Eds., New York: Humana Press, 2017, vol. 1601, p. 1. https://doi.org/10.1007/978-1-4939-6960-9_1