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Original Article

Synthesis, Structure and Biological Activity of (1*s*,9a*r*)-1*н*-1,2,3-Triazol-1-yl) Methyl) Octahydro-1*h*-Quinolizine Derivatives of Lupinine

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Abstract

Lupinine is an elementary representative of a large quinolizidine alkaloid group. Referring to a pharmacological action, lupinin has the bactericidal and low sedative effects. It also possesses the short-term anthelmintic and hypotensive properties. The curent research aimed to investigate synthesis methods and study of the hemorological, antimicrobial and cytotoxic activities of lupinine quinolizine alkaloid. The chemical modifications of lupinine molecules were performed with introduction of 1,2,3-triazole substituents for the hydroxymethylene group at C-1 position of quinolizine skeleton. Structure of the obtained compounds was determined by ^{1}H and ^{13}C NMR spectroscopy. The various correlation approaches of ^{1}H–^{1}H (COSY) and ^{1}H–^{13}C (HMBC and HSQC) spectroscopy were used for it. The evaluation results of the hemorheological, antimicrobial and cytotoxic activities of the obtained (1*S*,9a*R*)-1*H*-1,2,3-triazol-1-yl)methyl)octahydro-1*H*-quinolizine derivatives were demonstrated. Some compounds were identified and they are able to affect deformability of red blood cells and aggregation properties of blood. Patterns of the antimicrobial and cytotoxic activities depending on the structural features of the synthesized compounds were determined.

Keywords: Alkaloid Lupinine, Quinolizidine Derivatives, Hemorheological, Antimicrobial, Cytotoxic Activities, Incubation, Blood Hyperviscosity, *in vitro*

1. Introduction

Lupinine is an elementary representative of a large quinolizidine alkaloid group (1). Referring to a pharmacological action, lupinin has the bactericidal and low sedative effects. It also possesses the short-term anthelmintic and hypotensive properties. Many of the alkaloid lupinine derivatives are the valuable biologically active substances. It stimulated their comprehensive study, search for methods on the constructing of more complex structures and studying of structure-bioactivity relationship among its derivatives (2).

The results of the previous studies (3, 4) describes the pharmacological research of [(4-nitrobenzylidene)-imino] lupinine and [(2,4-dihydroxybenzylidene)-imino]lupinine compounds with the high antibiotic activity against plague and cholera microbes. A compound 11-[(gossypoliden)-imino] lupinine showed the high anti-AIDS activity. Among the known lupinine derivatives, its esters are most examined, and they have

the high antiviral, antitumor and hepatoprotective activities. Some lupinine esters demonstrated a local anesthetic effect and the anti-tuberculosis and anticholinesterase activities (5).

In view of the worldwide pandemic of COVID-19, the search issues of drugs affecting the rheological properties of blood are of current interest. The pathological changes in the rheological properties of blood play an important role to develop such diseases as the ischemic stroke, myocardial infarction, hypertension, bronchial asthma, diabetes, etc. (6, 7). The blood hyperviscosity syndrome was later discovered in the cardiovascular diseases (7), in many forms of cancer (8), the chronic obstructive pulmonary disease (COPD) (9) and other chronic diseases. To date the study of the clinical pathological processes determinates the indicators characterizing the state of microvasculature and rheological parameters of blood. In spite of the fact that a significant progress was achieved recently to study the mechanisms of the hemorheological disorders, the arsenal of pharmacological remedies is insignificant (10). The most effective drugs in this case are followed as pentoxifylline, clopidogrel, ticlopidine, aspirin, and hypolipidemic medicines (10). However, the incomplete efficacy and adverse effects of the synthetic drugs (dyspepsia, enterorrhagia, skin hemorrhages, leukopenia, thrombocytopenia, agranulocytosis) limits their use. A hemorheological crisis is one of the most common causes of the decompensation of the cerebral circulation. Hypercoagulation and hyperviscosity of blood are the basis for the systemic microcirculation disorders (11). Thus, derivatives of the natural compounds with the hemorheological, antimicrobial and cytotoxic activities are of particular interest (12). The modifying capabilities of a lupinine molecule structure permit to synthesize the analogous compounds and to study them for the similar types of activities. As a result, the interest in lupinine and its new derivatives is unabated. This paper demonstrates the results on synthesis of the new lupinine derivatives - (1*S*,9a*R*)-1*H*-1,2,3-triazol-1-yl) methyl) octahydro-1*H*-quinolisine compounds, and the possible effects on the blood rheological parameters and its antimicrobial and cytotoxic activities.

2. Materials and Methods

IR spectra were recorded on Vector-22 Fourier spectrometer with pressed KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on spectrometers of Bruker AV-400 (400 and 101 MHz, respectively) and Bruker DRX-500 (500 and 125 MHz, respectively). Compound spectra were recorded in $CDCl_3$, its signal (δ_C = 76.9 ppm) and residual signal of $CHCl_3$ (δ_H = 7.24 ppm) were used as an internal standard.

An atomic numbering of skeleton in structure was used to describe spectra. The specific rotations were measured on PolAAr 3005 polarimeter. The high resolution mass spectra were recorded on DFS ThermoScientific mass spectrometer (evaporator temperature 200–250°C, electron impact ionization, 70 eV). A melting point was determined on Mettler Toledo FP900 thermosystem.

Reaction progress control was monitored with TLC method on Sorbfil UV-254 plates using the systems such as chloroform, chloroform–ethanol, 10:1. Transmission is in an iodine chamber and in UV light. Reaction products were isolated with recrystallization or column chromatography on Acros silica gel (0.035–0.240 mm), eluents of $CHCl_3$; $CHCl_3$–EtOH, 100:1→10:1).

2.1. (*1R,9aR*)-Octahydro-1*H*-Quinolizine-1-yl)Methyl Methane-Sulfonate

A solution of methanesulfonyl chloride (4.8 g, 42 mmol) in 20 mL of CH_2Cl_2 was added by drops to solution cooled in ice bath of lupinine (3.54 g, 21 mmol) and triethylamine (6.36 g, 63 mmol) in CH_2Cl_2 (200 mL). Reaction mixture was stirred for 30 min when cooled to 0°C and 6 h at room temperature. Then it was washed with a saturated sodium chloride solution (2×20 ml) and dried over anhydrous $MgSO_4$. A drying agent was filtered. A solvent was distilled in a vacuum. Residue was chromatographed on a silica gel column (chloroform, chloroform-ethanol, 50:1). A yield was

4.84 g (93%). The cream crystals, melting point 57-58°C (from ether). $[\alpha]_D^{25}$ - 21.6 (*s* 1.4, $CHCl_3$). IR spectrum (KBr), ν, cm^{-1}: 1184, 1336 (OSO_2), 2740, 2757, 2798 (quinolizidine). ^1H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.12–1.26 (1H, m, H-2*a*); 1.28–1.51 (5H, m, H-2*e*,8*a,*8*e*,3*a*,7*a*); 1.54 (1H, m, H-9*a*); 1.59–1.77 (2H, m, H-3*e*,7*e*); 1.84–2.02 (5H, m, H-1,4*a*,6*a*,9*e*,9a); 2.73–2.80 (2H, m, H-4*e*,6*e*); 2.97 (3H, s, CH_3); 4.37 (1H, dd, *J* = 10.6, *J* = 9.8, H-10); 4.47 (1H, dd, *J* = 10.6, *J* = 5.3, H-10). ^{13}C NMR spectrum (101 MHz, $CDCl_3$), δ, ppm: 20.6 (C-3); 24.7; 25.4 C-7,8); 26.3 (C-2); 29.8 (C-9); 37.0 (CH_3); 38.0 (C-1); 56.8; 57.1 (C-4,6); 64.0 (C-9a); 69.5 (C-10). Mass spectrum, *m/z* ($I_{rel.}$, %): 248 (1), 247 (7), 153 (10), 152 (100), 150 (3), 98 (6). It was found that *m*/*z*: 247.1238 $[M]^+$. $C_{11}H_{21}NO_3S$. It was calculated that *m*/*z*: 247.1237.

2.2. (*1S,9aR*)-1-(Azidomethyl)Octahydro-1*H*-Quinolizine

A mixture of compound (**2**) (4.84 g, 20 mmol) and sodium azide 3.44 g (53 mmol) in DMF (50 ml) was stirred at 70°C for 5 h (TLC control). A reacting mass was poured in Petri dish to evaporate freely a solvent in air. A residue was dissolved in CH_2Cl_2, washed with a saturated sodium chloride solution and then dried over anhydrous $MgSO_4$. A drying agent was filtered, a solvent was distilled in vacuum, and then a residue was chromatographed on a silica gel column (chloroform-ethanol, 50:1). The yield was 2.33 g (60%). It is a light yellow and mobile liquid. $[\alpha]_D^{26}$ -29.85 (*s* 2.4, chloroform). IR spectrum, ν, cm^{-1}: 1269, 2096 (N≡N), 2744, 2762, 2804 (quinolizidine). ^1H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*,Hz): 1.12–1.26 (1H, m, H-2*a*); 1.30–1.57 (6H, m, H-8*a,*8*e*,9*a*,9*e*,3*a*,7*a*); 1.58–1.76 (3H, m, H-2*e*,3*e*,7*e*); 1.80–1.99 (4H, m, H-1,4*a*,6*a*,9*a*); 2.72-2.82 (2H, m, H-4*e*,6*e*); 3.42 (1H, dd, *J* = 12.6, *J* = 9.6, CH_2-10); 3.54 (1H, dd, *J* = 12.6, *J* = 5.3, CH_2-10). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 20.7 (C-3); 24.9 (C-8); 25.4 (C-7); 27.3 (C-2); 29.6 (C-9); 38.2 (C-1); 50.4 (C-10); 56.8; 57.2 (C-4,6); 64.3 (C-9a). Mass spectrum, *m/z* ($I_{rel.}$, %): 194 (2), 153 (10), 152 (100), 137 (7), 136 (5), 98 (12), 84 (7), 83 (9), 82 (6), 55 (10), 41 (14). It was found that *m*/*z*: 194.1528 $[M]^+$. $C_{10}H_{18}N_4$. It was calculated that *m*/*z*: 194.1526.

2.3. Synthesis of Compounds (Compound 5a-e) (a General Procedure)

A mixture of azide 3 (0.29 g, 1.5 mmol), a substituted acetylene (Compound 4a-e) (1.35 mmol), $CuSO_4{\times}5H_2O$ (0.017 g, 0.0675 mmol) and sodium ascorbate (0.013 g, 0.0675 mmol) in DMF (4 ml) was stirred at 75°C for 4-6 h (TLC control). A residue formed at cooling was filtered, washed with hexane and dried. Then triazoles (Compound 5a-e) were obtained. In order to isolate triazoles (5a–e), a solvent was distilled in a vacuum, a residue was chromatographed on a silica gel column (eluent: chloroform, mixture of chloroform and ethanol, 100:1 → 10:1).

2.4. (*1S,9aR*)-1-{[4-(4-Methoxyphenyl)-1*H*-1,2,3-Triazol-1-yl]Methyl}Octahydro-1*H*-Quinolizine (Compound 5a).

The yield was 0.35 g (83%). It is white crystals, m.p. 177–178°C (from ethyl acetate). $[\alpha]_D^{26}$ -16.9 (*s* 0.8, chloroform). IR spectrum, ν, cm^{-1}: 829, 920, 1443, 1458, 1498, 1560, 1618, 3097 (C=C, C=N); 1008, 1132, 1246 (C-O); 2761, 2804 (quinolizidine). ^1H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.17–1.40 (3H, m, H-2*a*,2*e*,8*a*); 1.40–1.64 (5H, m, H-8*e*,9*a*,9*e*,3*a*,7*a*); 1.73–1.90 (2H, m, H-3*e*,7*e*); 1.92-2.05 (2H, m, H-4*a*,6*a*); 2.06-2.10 (1H, m, H-9a), 2.22-2.26 (1H, m, H-1); 2.83-2.88 (2H, m, H-4*e*,6*e*); 3.81 (3H, s, OCH_3); 4.54 (1H, dd, *J* = 13.8, *J* = 5.5, H-10); 4.60 (1H, dd, *J* = 13.8, *J* = 12.5, H-10); 6.92 (2H, d, *J* = 8.6, H-3″,5″); 7.61 (1H, s, H-5′); 7.73 (2H, d, *J* = 8.6, H-2″,6″). ^{13}C NMR spectrum (101 MHz, $CDCl_3$), δ, ppm: 20.5 (C-3); 24.7; 25.4 (C-7,8); 26.1 (C-2); 29.5 (C-9); 39.1(C-1); 48.4 (C-10); 55.2 OCH_3); 56.9; 57.2 (C-4,6); 64.3 (C-9a); 114.1 (C-3″,5″); 119.3 (C-5′); 123.3 (C-1″); 126.8 (C-2″,6″); 147.2 (C-4′); 159.4 (C-4″). Mass spectrum, *m/z* (I_{rel}, %): 328 (1), 327 (12), 226 (49), 152 (42), 151 (100), 150 (66), 138 (18), 137 (14), 136 (33), 111 (18), 96 (17), 83 (25), 41 (150). It was found that *m*/*z*: 326.2100 $[M]^+$. $C_{19}H_{26}N_4O$. It was calculated that *m*/*z*: 326.2101.

2.5. (*1S,9aR*)-1-{[4-(*m*-tolyl)-1*H*-1,2,3-Triazol-1-yl]Methyl}Octahydro-1H-Quinolizine (Compound 5b). The yield was 0.52 g (80%). It is white crystals, m.p. 141–142°C (from ethyl acetate). $[\alpha]_D^{26}$ -13.8 (*s* 1.0, chloroform). IR spectrum, ν, cm^{-1}: 694, 791, 846, 1443, 1464, 1487, 1614, 3122 (C=C, C=N); 2763, 2804 (quinolizidine). ^{1}H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.20–1.40 (3H, m, H-2*a,e*,8*a*); 1.41–1.63 (5H, m, H-8 *e*,9*a*,9*e*,3*a*,7*a*); 1.74–1.91 (2H, m, H-3*e*,7*e*); 1.94-2.02 (2H, m, H-4*a*,6*a*); 2.06-2.09 (1H, m, H-9a), 2.22-2.26 (1H, m, H-1); 2.37 (3H, s, CH_3); 2.83-2.88 (2H, m, H-4*e*,6*e*); 4.56 (1H, dd, *J* = 13.8, *J* = 5.8, H-10); 4.61 (1H, dd, *J* = 13.8, *J* = 11.2, H-10); 7.11 (1H, d, *J* = 7.5, H-4″); 7.27 (1H, t, *J* = 7.5, H-5″); 7.58 (1H, dd, *J* = 7.5, *J* = 1.6, H-6″); 7.66 (1H, s, H-5′); 7.72 (1H, d, *J* = 1.6, H-2″). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 20.5 (C-3); 21.3 (CH_3); 24.7; 25.4 (C-7,8); 26.2 (C-2); 29.6 (C-9); 39.1 (C-1); 48.5 (C-10); 56.91; 57.2 (C-4,6); 64.3 (C-9a); 120.0 (C-5′); 122.7; 126.2; 128.5; 128.6 (C-2″,4″,5″,6″); 130.5 (C-1″); 138.3 (C-3″); 147.5 (C-4′). Mass spectrum, *m/z* (I_{rel}, %): 312 (1), 311 (9), 310 (42), 152 (28), 151 (100), 150 (52), 138 (15), 136 (35), 83 (20). It was found that *m/z*: 310.2155 $[M]^+$. $C_{19}H_{26}N_4$. It was calculated that *m/z*: 310.2152.

2.6. (*1S,9aR*)-1-[4-Phenyl-1*H*-1,2,3-Triazol-1-yl)Methyl]octahydro-1*H*-quinolizine (Compound 5c)

The yield was 0.3 g (75%). It is a white powder, m.p. 196–197°C (decomp.). $[\alpha]_D^{26}$ - 19.7 (*s* 0.8, chloroform). IR spectrum, ν, cm^{-1}: 694, 766, 835, 1441, 1466, 1485, 1505, 1612, 3120 (C=C, C=N), 2763, 2804 (quinolizidine). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.17–1.40 (3H, m, H-2*a*, 2*e*,8*a*); 1.41–1.64 (5H, m, H-8*e*,9*a*,9*e*,3*a*,7*a*); 1.73–1.92 (2H, m, H-3*e*,7*e*); 1.94-2.04 (2H, m, H-4*a*, H-6*a*); 2.06-2.11 (1H, m, H-9a), 2.21-2.25 (1H, m, H-1); 2.82-2.88 (2H, m, H-4*e*,6*e*); 4.55 (1H, dd, *J* = 13.6, *J* = 5.8, H-10); 4.62 (1H, dd, *J* = 13.6, *J* = 12.0, H-10); 7.29 (1H, t, *J* = 7.6, H-4″); 7.39 (dd, 2H, *J* = 7.4, 7.6, H-3″,5″); 7.70 (1H, s, H-5′), 7.80 (2H, d, *J* = 7.4, H-2″,6″). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 20.5 (C-3); 24.7; 25.3 (C-7,8); 26.3 (C-2); 29.5 (C-9); 39.1 (C-1); 48.5 (C-10); 56.9; 57.1 (C-4,6); 64.2 (C-9a); 120.0 (C-5′); 125.6 (2C, *o*-C_6H_5); 127.9 (C, *p*-C_6H_5); 128.7 (2C, *m*-C_6H_5); 130.7 (C, C_6H_5); 147.4 (C-4′). Mass spectrum, *m/z* (I_{rel}, %): 298 (1), 297 (8), 296 (38), 152 (42), 151 (100), 150 (59), 138 (22), 137 (11), 136 (32), 116 (16), 111 (24), 110 (19), 96 (19), 83 (36), 41 (36). It was found that *m/z*: 296.1995 $[M]^+$. $C_{18}H_{24}N_4$. It was calculated that *m/z*: 296.1996.

2.7. (*1S,9aR*)-{1-[(4-(Hydroxymethyl)-1,2,3-Triazol-1-yl]Methyl}Octahydro-1*H*-Quinolizine (Compound 5d)

The yield was 0.35 g (77%). It is a white powder, m.p. 119–122°C. $[\alpha]_D^{25}$ - 24.2 (*s* 0.8, chloroform). IR spectrum, ν, cm^{-1}: 796, 1443, 1466, 3116 (C=C, C=N), 1022, 1037, 1045, 1130 (C-O), 2736, 2759, 2800 (quinolizidine), 3310 (OH). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.17–1.37 (3H, m, H-2*a*,2*e*,8*a*): 1.38–1.62 (5H, m, H-8*e*,9*a*,9*e*,3*a*,7*a*); 1.72–1.86 (m, 2H, H-3*e*,7*e*); 1.90-2.04 (2H, m, H-4*a*,6*a*); 2.06-2.14 (1H, m, H-9a), 2.18-2.22 (1H, m, H-1); 2.81-2.85 (m, 2H, H-4*e*,6*e*); 3.32 (1H, br. s, OH); 4.48 (1H, dd, *J* = 13.3, *J* = 5.8, H-10); 4.50 (1H, dd, *J* = 13.3, *J* = 10.9, H-10); 4.75 (2H, s, $\underline{CH_2}$OH); 7.49 (1H, s, C-5′). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 20.5 (C-3); 24.7; 25.3 (C-7,8); 26.2 (C-2); 29.5 (C-9); 39.1 (C-1); 48.5 (C-10); 56.4 (CH_2OH); 56.8; 57.1 (C-4,6); 64.2 (C-9a); 122.1 (C-5′); 147.4 (C-4′). Mass spectrum, *m/z* ($I_{rel.}$, %): 251 (4), 250 (20), 152 (35), 151 (100), 150 (55), 136 (40), 111 (23), 110 (18), 96 (19), 83 (39), 55 (10), 41 (15). It was found that *m/z*: 250.1786 $[M]^+$. $C_{13}H_{22}N_4O$. It was calculated that *m/z*: 250.1788.

2.8. (*1S,9aR*)-1-{[4-(2-Hydroxypropan-2-yl)-1,2,3-Triazol-1-yl]Methyl}Octahydro-1*H*-Quinolizine (Compound 5e). The yield was 0.38 g (81%). It is a white crystalline powder, m.p. 137–139°C. $[\alpha]_D^{25}$ - 20.4 (*s* 0.8, chloroform). IR spectrum, ν, cm^{-1}: 748, 837, 858, 1442, 1460, 1538, 3128 (C=C, C=N), 1056, 1064, 1107, 1126, 1144 (C-O), 2767, 2808 (quinolizidine), 3423 (OH). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz); 1.18–1.37 (3H, m, H-2*a*,2*e*,8*a*); 1.38–1.58 (5H, m, H-8*e*,9*a*,9*e*,3*a*,7*a*); 1.60 (6H, s, 2×CH_3); 1.72–

1.90 (2H, m, H-3*e*,7*e*); 1.91–2.05 (2H, m, H-4*a*,6*a*); 2.05–2.12 (1H, m, H-9a), 2.15–2.19 (1H, m, H-1); 2.67 (1H, br. s, OH); 2.80-2.86 (2H, m, H-4*e*,6*e*); 4.46 (1H, dd, *J* = 13.2, *J* = 5.6, H-10); 7.37 (1H, s, C-5′). ^{13}C NMR spectrum (125 MHz, $CDCl_3$), δ, ppm: 22.0 (C-3); 26.2; 26.9 (C-7,8); 27.8 (C-2); 31.1 (C-9); 31.9 (2×CH_3); 40.7 (C-1); 49.9 (10); 58.4; 58.7 (C-4,6); 65.8 (C-9a); 69.9 (C-6′); 120.9 (C-5′); 156.8 (C-4′). Mass spectrum, *m/z* (I_{rel}, %): 279 (3), 278 (13), 152 (33), 151 (100), 150 (54), 136 (35), 111 (17), 110 (14), 83 (28), 41 (15). It was found that *m*/z: 278.2097 $[M]^+$. $C_{15}H_{26}N_4O$. It was calculated that *m*/*z*: 278.2101.

The hemorheological effects of the tested samples of substances were studied under conditions *in vitro*. Hyperviscosity syndrome under *in vitro* conditions was reproduced with blood incubation at 43.0°C for 60 min (13). Blood viscosity was measured on Brookfield DV2T rotary viscometer at some spindle speeds (40, 20, 12, 8, 6, 4, 2 s^{-1}).

The laboratory animals (male Wistar rats) had the blood sampling. Then an initial blood viscosity was determined. Blood samples were incubated with test substances at 43.0°C for 60 min. Then the test parameters were measured. Blood was incubated with the test objects dissolved in DMSO. A final concentration of substances was 10^{-4} g/ml blood. Blood samples with added DMSO solvent in an equivolume amount were as a control. Incubation of blood for 1 h under these conditions was accompanied by the stationary weight control (13).

Cultures were cultivated in a liquid medium (pH 7.3 ± 0.2) at a temperature from 30 to 35°C for 18–20 hours. The cultures were diluted (1:1000) in a sterile 0.9% isotonic sodium chloride solution, added 1 ml each to dishes with the special elective nutrient media for the studied test-strains. Then it was inoculated with using a lawn method. After drying, wells (6.0 mm) were formed on an agar surface, then a solution of the tested samples, gentamicin and nystatin were added. Ethyl alcohol was used in the control in equivolume amounts. The tested samples were studied in the amount of 1 μg. Inoculations were incubated at 37°C. The growing cultures were counted after 24 h.

The antimicrobial activity of the samples was evaluated with diameter of the growth retardation zones of test-strains (mm). The diameter of growth retardation zones is less 10 mm. The continuous growth in dish was evaluated as absence of the antibacterial activity, 10-15 mm – a weak activity, 15-20 mm – a moderate activity and more 20 mm – a high activity. Each sample was tested in three parallel experiments.

Cytotoxicity was evaluated in a survival test of marine crustacean larvae *Artemia salina* (Leach). The experiments were made on 2-day-old larvae under cultivation conditions *in vitro*. Larvae were grown with adding the marine crustacean eggs of *Artemia salina* (Leach) into artificial sea water and incubated for 48 h at 37°C. A test portion of each sample was dissolved in ethanol (2 ml). Then 500 μl (3 parallels), 50 μl (3 parallels) and 5 μl (3 parallels) were taken from this solution.

After ethanol evaporation, 5 ml of artificial sea water was added to each bottle. Thus, if an initial sample weight was 2 mg, the final sample concentrations were 100 μg/mL, 10 μg/mL and 1 μg/mL, respectively, of each concentration in 3 replicates. Ten marine crustacean larvae of *Artemia salina* (2 days old) were placed in each sample bottle using a Pasteur pipette.

After that, all bottles were kept at room temperature in the light for 24 h. After 24 hours, the surviving and dead larvae were counted. Then upper and lower toxic limits were used to calculate the half toxic dose of each sample. A reference drug was 13-dimethylamino-1,10β-epoxy-5,7α,6,11β(H)-guai-3,4-en-6,12-olide hydrochloride (substance of drug "Arglabin"), having an antitumor activity.

2.9. Statistical Analysis

The statistical processing was made by the parametric statistics methods using calculation of an arithmetic mean and standard error.

3. Results and Discussion

Reaction of lupinine (Figure 1, compound 1) and methanesulfonyl chloride (2 equiv.) with triethylamine (3 equiv.) in a methylene chloride at cooling forms a (1R,9a*R*)-octahydro-1*H*-quinolizine-1-yl) methyl methane-sulfonate (Figure 1, compound 2). The yield is 93%. The treatment of compound 2 with NaN_3 in DMF medium under heating resulted in formation of 1-(azidomethyl) octahydro-1*H*-quinolisine (Figure 1, compound 3) isolated in 61% yield with the column chromatography on silica gel.

Reaction of lupinilazide (Figure 1, compound 3) with arylalkynes (4-methoxyphenylacetylene (Figure 2. Compound 4a), *m*-tolylacetylene (Figure 2. compound 4b), phenylacetylene (Figure 2. compound 4c)) good proceeded in DMF medium with $CuSO_4 \times 5H_2O$ and NaAsc under heating to 75°C (TLC control). The (1*S*,9a*R*)-1*H*-1,2,3-triazol-1-yl) methyl) octahydro-1*H*-quinolizines (Figure 2. compound 5a-c) containing the aryl substituent at position C-4 of 1,2,3-triazole cycle were isolated after column chromatography on silica gel.

Reaction of lupinine azide (Figure 1. compound 3) with alcohols containing a terminal acetylene group (propargyl alcohol (Figure 2. compound 4d) or 2-methylbut-3-in-2-ol (Figure 2. Compound 4e)) synthesized {1-[4-(hydroxyalkyl)-1,2,3-triazol-1-yl]methyl} octahydro-1*H*-quinolizines (5d, e) (77-81% yield) (Figure 2). Introduction of the terminal acetylenes (Figure 2. Compound 4d, e) into reaction gives possibility for further transformations involving the functional groups.

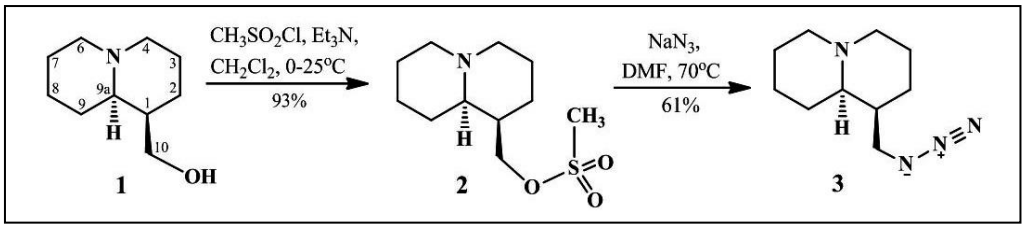

Figure 1. Synthesis of lupinine azide

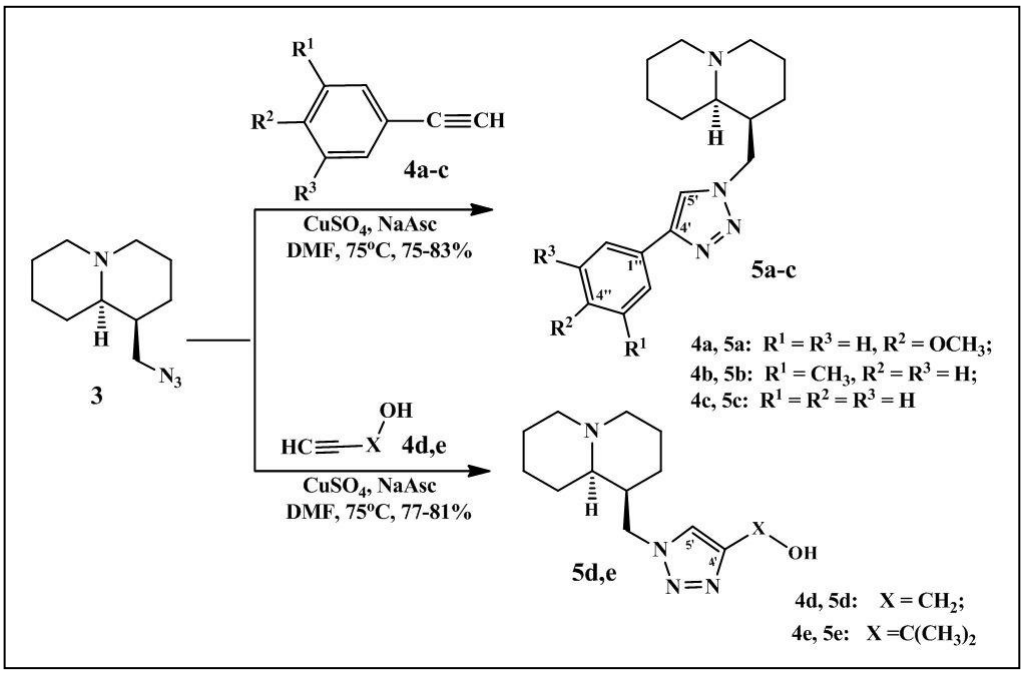

Figure 2. Reaction of lupinine azide [3] with aryl (4a-c) and terminal (4d, e) alkynes

Composition and structure of the synthesized compounds (Figure 2. compound 2, 3, 5a-e) were justified with IR, ^{1}H and ^{13}C NMR – spectroscopy and mass spectrometry. An azide substituent in compound [3] structure in the IR spectrum is observed as an intense absorption band at 2096 cm^{-1}.

^{1}H and ^{13}C NMR spectra of the synthesized quinolizine 1,2,3-triazoles include a special signal set for quinolizine skeleton and a relevant substituent. The wide multiplet signals with an integral intensity of 8H take place in a high-field region (δ 1.17–1.70 ppm). They include protons of lupinine skeleton of the axial and equatorial orientations (H-2*a*,*e*,8*a*,*e*,9*a*,*e*,3*a*,7*a*).

A multiplet signal (δ 1.70-1.92 ppm) belongs to the equatorially oriented protons H-3,7. Then, the resonating is observed in axial protons 4,6 (δ 1.88-2.08 ppm), node proton 9a (δ 2.05-2.18 ppm) and proton C-1 (δ 2.18-2.30 ppm). The equatorial-oriented protons 4,6 are represented as a narrow multiplet in region (δ 2.80-2.88 ppm). Methylene protons of H-10 resonate as two doublets in region of δ 4.51-4.65 ppm.

Proton of the 1,2,3-triazole cycles in ^{1}H NMR spectra of compounds (Figure 2. Compound 5a-e) responds to a singlet signal in region of δ 7.37-7.71 ppm. Carbon atoms of a triazole cycle in ^{13}C NMR spectra correspond to signals at 119.3-122.4 (C-5) and 146.2-156.8 ppm (C-4) of doublet and singlet, respectively. The recording spectra are in JMOD mode. These data confirm that result of CuAAC reaction forms the 1,4-disubstituted 1*H*-1,2,3-triazoles (13).

Mass spectra of all compounds include the molecular ion peaks with a different intensity. A peak of fragment ion of $C_{10}H_{17}N$ (150-151 c.u) is observed in spectra of all synthesized quinolizidinotriazoles (Figure 2. Compound 5a-e). It is correspond to the breaking of molecule at C-10 atom of a quinolizidine skeleton.

Blood viscosity is defined with a state of the macrorheological parameters (volume concentration of blood cells, i.e. hematocrit), with plasma viscosity (depends on type and concentration of proteins) and with parameters of cellular rheology, i.e. deformability (depends on viscosity of membrane and internal contents) and with erythrocyte aggregation.

Blood viscosity is able to increase as a result of raising in hematocrit, plasma viscosity (due to increase in fibrinogen and other proteins with high molecular mass in it), aggregation, and decrease in deformability of erythrocytes (due to increase in viscosity of contents of erythrocytes and / or viscosity of cell membranes) (14). Parameters of blood viscosity at high shear rates are predominantly determined by deformability of erythrocytes, and blood viscosity at low shear rates mainly depends on aggregation of erythrocytes (15).

The high blood viscosity syndrome has no the specific evidences. Thus, it can be diagnosed only with the special laboratory tests (15). The research experiments of the hemorheological activity of blood samples determined that the incubation process for 60 min at 43.0°C leads to an actual increase in blood viscosity at various spindle speed (2 s^{-1} to 40 s^{-1}). It testifies formation of the blood hyperviscosity.

Among our tested samples, only 1-{[4-(4-methoxyphenyl)-1*H*-1,2,3-triazolyl-1-yl]-methyl}octahydro-1*H*-quinolizine (compound 5a) and 1-[(4-*m*-tolyl-1*H*-1,2,3-triazol-1-yl)methyl]octahydro-1*H*-quinolizine (compound 5b) had the ability to reduce blood viscosity in model of blood hyperviscosity *in vitro*. Table 1 demonstrates the screening results of 1-{[4-(4-methoxyphenyl)-1*H*-1,2,3-triazolyl-1-yl]-methyl}octahydro-1*H*-quinolizine (compound 5a) and 1-[(4-*m*-tolyl-1*H*-1,2,3-triazol-1-yl)methyl]octahydro-1*H*-quinolizine (compound 5b) on the hemorheological activity in model of blood hyperviscosity *in vitro*.

Increase in blood viscosity was observed in the whole tested range of shear rates during the blood incubation. The tested samples of (compound 5a) and (compound 5b) demonstrated the hemorheological activity limiting the blood viscosity growth in the whole tested range of shear rates. It can be assumed that samples of (compound 5a) and (compound 5b) are able to impact on deformability of erythrocytes and their aggregation properties.

As a result of the search for the potential syndrome correctors of the increased blood viscosity among the tested compounds, the most active substances were 1-{[4-(4-methoxyphenyl)-1*H*-1,2,3-triazolyl-1-yl]-methyl}octahydro-1*H*-quinolizine (compound 5a) and 1-[(4-m-tolyl-1*H*-1,2,3-triazol-1-yl)methyl]octahydro-1*H*-quinolizine (compound 5b).

The antimicrobial activity of the above mentioned samples was studied on strains of gram-positive bacteria of *Staphylococcus aureus*, *Bacillus subtilis*, to gram-negative strains of *Escherichia coli*, *Pseudomonas aeruginosa* and to yeast fungus of *Candida albicans* with using the diffusion into agar wells.

Reference drugs are Gentamicin for bacteria and Nystatin for yeast fungus *C. albicans*. The tested results of the antimicrobial activity of samples are demonstrated in table 2.

Table 1. Impact of samples of (compound 5a) and (compound 5b) on blood viscosity (mPa*s) at some spindle speeds (shear rates) in model of blood hyperviscosity *in vitro*

Tested parameter	blood viscosity (mPa*s) at some spindle speeds (shear rates), revolution per minute (s^{-1})						
	40	20	12	8	6	4	2
Initial viscosity	5.15	5.61	6.03	6.71	7.36	8.2	10.88
Blood viscosity after 1 h of incubation at 43°C, adding DMSO solvent to a sample (control)	6.66	7.48	8.67	9.87	10.76	12.61	17.48
Blood viscosity after 1 h of incubation at 43°C, added to sample (5a) dissolved in DMSO at concentration of 10^{-4} g/ml	5.97	6.73	7.74	8.89	9.63	11.27	15.64
Blood viscosity after 1 h of incubation at 43°C, added to sample (5b) dissolved in DMSO at concentration of 10^{-4} g/ml	6.00	6.54	7.30	8.09	8.51	9.74	13.95

Table 2. The antimicrobial activity of samples of lupinine (1) and its derivatives (2,3,5a-e)

No.	Compound	*S. aureus*	*Bac. subtilis*	*E. coli*	*Ps. aeruginosa*	*C. albicans*
1	(1)	18 ±0.2	16±0.2	23 ±0.1	15 ±0.2	-
2	(2)	16±0.1	-	18±0.1	-	-
3	(3)	17 ±0.1*	17±0.2	21±0.1	14±0.1	13±0.2
4	(5a)	18 ±0.2	15±0.2	16±0.1	-	-
5	(5b)	24±0.1	18±0.2	18±0.1	12±0.2	14±0.1
6	(5c)	20±0.1	16±0.2	17±0.2	-	13±0.2
7	(5d)	19 ±0.2	16±0.2	16±0.1	-	13±0.2
8	(5e)	17±0.2	16±0.1*	15±0.1	-	-
Gentamicin		24 ± 0.1	21 ± 0.2	26 ± 0.1	27±0.1	-
Nystatin		-	-	-		21 ± 0.2

Note -*- reliability of differences $P<0.05$ compared to a comparison group

It was found that Samples of lupinine (compound 1), (1*S*,9a*R*)-1-(azidomethyl)octahydro-1*H*-quinolizine (compound 3) have a high antibacterial activity against gram-negative test-strain *Escherichia coli*, and the moderate antimicrobial activity against gram-positive test-strains of *Staphylococcus aureus* and *Bacillus subtilis*. Samples of (1*S*,9a*R*)-1-{[4-(*m*-tolyl)-1*H*-1,2,3-triazol-1-yl]methyl}octahydro-1*H*-quinolizine (compound 5b) and (1*S*,9a*R*)-1-[4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl]octahydro-1*H*-quinolizine (compound 5c) have a high antimicrobial activity against gram-positive test-strain *Staphylococcus aureus* and a moderate antibacterial activity against strains of *Bacillus subtilis* and *Escherichia coli*. Compounds of (1*R*,9a*R*)-octahydro-1*H*-quinolizine-1-yl)methyl methanesulfonate (compound 2) and (*1S,9aR*)-1-{[4-

(4-methoxyphenyl)-1*H*-1,2,3-triazole-1-yl]methyl}octahydro-1*H*-quinolizine (compound 5a) have a moderate antibacterial activity against gram-positive test-strain *Staphylococcus aureus* and gram-negative test-strain *Escherichia coli*.

Substances of (*1S,9aR*)-{1-[(4-(hydroxymethyl)-1,2,3-triazol-1-yl]methyl}octahydro-1*H*-quinolizine (compound 5d) and (*1S,9aR*)-1-{[4-(2-hydroxypropan-2-yl)-1,2,3-triazol-1-yl]methyl}octahydro-1*H*-quinolizine (compound 5e) have a moderate antibacterial activity against gram-positive strains of *Staphylococcus aureus* and *Bacillus subtilis*. However, a sample (*1S,9aR*)-{1-[(4-(Hydroxymethyl)-1,2,3-triazol-1-yl]methyl}octahydro-1H-quinolizine (compound 5d) has a moderate activity against gram-negative strain *Escherichia coli*.

The tested results on the cytotoxic activity of lupinine samples against larvae of marine crustaceans *Artemia salina* (Leach) under cultivation conditions *in vitro* are demonstrated in table 3.

Table 3. The cytotoxic activity of samples of lupinine (1) and its derivatives (2,3,5a-e)

Compound	Concentration µg/ml	Number of surviving larvae			LD_{50}, µg/ml	Activity
		Parallel 1	Parallel 2	Parallel 3		
(1)	1	8	8	8	62.7	yes
	10	5	5	5		
	100	4	4	4		
(2)	1	9	9	9	77.3	yes
	10	7	7	6		
	100	5	5	5		
(3)	1	9	9	8	68.5	yes
	10	7	7	6		
	100	5	4	5		
(5a)	1	9	9	9	75.6	yes
	10	7	6	6		
	100	5	5	4		
(5b)	1	8	8	8	58.1	yes
	10	5	4	5		
	100	3	4	4		
(5c)	1	8	9	9	71.4	yes
	10	6	7	6		
	100	4	5	4		
(5d)	1	10	9	9	83.6	yes
	10	7	6	6		
	100	5	5	4		
(5e)	1	9	8	8	87.1	yes
	10	6	6	5		
	100	4	4	4		
Reference drug: 13-dimethylamino-1,10β-epoxy-5,7α,6,11β(H)-guai-3,4-ene-6,12-olide hydrochloride	1	8	7	7	20.6	
	10	5	4	4		
	100	4	2	2		

Table 3 demonstrates that samples of lupinine (compound 1), (*1R,9aR*)-octahydro-1*H*-quinolizine-1-yl)methyl methanesulfonate (compound 2), (*1S,9aR*)-1-(azidomethyl)octahydro-1*H*-quinolizine (compound 3), (*1S,9aR*)-1-{[4-(4-methoxyphenyl)-1*H*-1,2,3-triazol-1-yl]methyl}octahydro-1*H*-quinolizine (compound 5a), (*1S,9aR*)-1-{[4-(*m*-tolyl)-1*H*-1,2,3-triazol-1-yl]methyl}octahydro-1*H*-quinolizine (compound 5b), (*1S,9aR*)-1-[4-phenyl-1*H*-1,2,3-triazol-1-yl)methyl]octahydro-1*H*-quinolizine (compound 5c), (*1S,9aR*)-{1-[(4-(hydroxymethyl)-1,2,3-triazol-1-yl]methyl}octahydro-1*H*-quinolizine (compound 5d), (*1S,9aR*)-1-{[4-(2-hydroxypropan-2-yl)-1,2,3-triazol-1-yl]methyl}octahydro-1*H*-quinolizine (compound 5e) have the cytotoxic activity against the larvae of marine crustaceans *Artemia salina* (Leach). However, the cytotoxic activity of samples of lupinine (compound 1) and (*1S,9aR*)-1-{[4-(*m*-tolyl)-1*H*-1,2,3-triazol-1-yl]methyl}octahydro-1*H*-quinolizine (compound 5b) is higher than the above samples have.

Thus, quinolizine alkaloid lupinine with introduction of a 1,2,3-triazole heterocyclic substituent at C-10 position of an alkaloid was first modified. As the research result, 5 novel (*1S,9aR*)-1*H*-1,2,3-triazol-1-yl)methyl)octahydro-1*H*-quinolizine and mesylate and azide derivatives of lupinine were synthesized.

The substituent at position C-4 of a triazole cycle was demonstrated to have a significant effect on the hemorheological, antimicrobial and cytotoxic activities of a new group of lupinine derivatives. As a result, it was found that the introduced fragments are able to provide the additional ligand-receptor interactions of the potentially biologically active compounds. Thereby they are able to change the selectivity of the biological

Authors' Contribution

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Acquisition of data: S. Zh. N.
Analysis and interpretation of data: O. A. N.
Drafting of the manuscript: Z. M. M.
Critical revision of the manuscript for important intellectual content: S. D. F.
Statistical analysis: R. B. S.
Administrative, technical, and material support: Z. T. S.

Conflict of Interest

The authors declare that they have no conflict of interest.

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