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SYNTHESIS, STRUCTURE AND BINDING AFFINITY OF A NOVEL 1,2,3-TRIAZOLYL-CARVONE WITH BOVINE SERUM ALBUMIN

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On the basis of natural L-carvone monoterpenoide, a method for the synthesis of (5*R*)-2-methyl-5-(3-{4-[(azepan-1-yl)methyl]-1*H*-1,2,3-triazol-1-yl}prop-1-en--2-yl)cyclohex-2-en-1-one has been developed. The reaction conditions providing high yields of the target products were optimized. The structure of the synthesized compound has been confirmed and the physicochemical constants have been determined. The nature of the interaction and the thermodynamic characteristics of the 1,2,3-triazolyl-carvone with serum albumin (BSA) have been also determined. It has been shown that hydrophobic interactions are the predominant intermolecular forces stabilizing 1,2,3-triazolyl-carvone–BSA system. The distance between donor (BSA) and acceptor (1,2,3-triazolyl-carvone) has been calculated using Förster's fluorescence energy transfer theory.

Keywords: 1,2,3-triazolyl-carvone, antibacterial activity, fluorescence spectroscopy, binding parameters, albumin.

Introduction. The gradual increase in resistance rates of several important pathogens (*Staphylococcus*, *Enterococcus*, *Escherichia coli, Klebsiella pneumonia* etc.) against antibiotics causes a serious problem for clinical medicine [1–3]. One of the methods to reduce the resistance in pathogenic bacteria is the development the efficiency of antibiotics. Terpenoids, also referred to as terpenes, are the largest group of natural compounds that have biological activities and are used for the treatment of human diseases [4]. Development in pathogenic microorganisms' resistance actually is not mentioned even in prolonged use of terpene containing medicines such as Biopin, Abisil etc. This is undoubtedly an advantage and makes this class of compounds promising in increasing the efficiency of drugs.

Azoles, especially 1,2,4- and 1,2,3-triazoles, can be used for this purpose. A large number of 1,2,4-triazoles exhibit important therapeutic activities such as antimicrobial, anticancer, antioxidant, antifungal [5–7]. Furthermore, 1,2,4-triazoles have been incorporated into a wide variety of therapeutically effective drug candidates as antiviral agent (Ribavirin), antifungal agent (Fluconazole, Itraconazole, Voriconazole), antimigraine agent (Rizatriptan) [8].

1,2,3-Triazole derivatives are relatively little studied. Biological studies have shown that particular representatives of 1,2,3-triazoles exhibit antibiofilm, antineoplastic, anticancer, antiplasmodial and antibacterial activities [9–12]. Thus, there is

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a need to explore these pharmacophores for the development of novel molecules with different activities.

The aim of this article is to synthesize a novel triazole derivative with a fragment of natural product, combined with heterocyclic pharmacophore fragment 1,2,3-triazole and to study of the binding affinity of this compound with drug transporting protein – serum albumin.

Materials and Methods.

Synthesis and Structure of 1,2,3-triazolyl-carvone. Reagents and solvents for the synthesis were of reagent grade and were used as such or distilled prior to use. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE 400 *MHz* spectrometer in $CDCl_3$. Chemical shifts (δ) in *ppm* are reported as quoted relative to the residual signals of chloroform-*d* (7.26 for ^{1}H NMR and 77.16 for ^{13}C NMR) as internal references.

ESI–MS spectra were measured with a micrOTOF Bruker Daltonics instrument. TLC analysis was performed on Silufol UV-254 plates. Compound **2** (see Scheme) was synthesized according to the procedure described in [13]. Melting point was determined on Boetius micro-heating stage and SMP10.

(5R)-5-(3-Chloroprop-1-en-2-yl)-2-methylcyclohex-2-en-1-one (2). B.p. 110–116°*C*/1 *Torr*, $n_D^{20} = 1.5285$, $[a]_D^{20} = 44.05$ (C 2.17 CH_2Cl_2).

Method of Synthesis of (5R)-2-methyl-5-(3-{4-[(azepan-1-yl)methyl]-1H--1,2,3-triazol-1-yl}prop-1-en-2-yl)cyclohex-2-en-1-one. 369 *mg* (2 *mmol*) of (5*R*)-5-(3-chloroprop-1-en-2-yl)-2-methylcyclohex-2-en-1-one, 2 *mL* of acetonitrile, 156 *mg* (2.4 *mmol*) of sodium azide are placed in a round-bottomed 5 *mL* flask and heated with stirring for 10 *h* (60°*C*). After cooling, 242 *mg* (2.4 *mmol*) of triethylamine, 19 *mg* (0.1 *mmol*) of CuI and 2.4 *mmol* of N-propargylazepane are added to the resulting mixture. The whole is heated then for 10 *h* at 60°*C* and the solvent is distilled off. The cooled mixture is dissolved in benzene, filtered and hexane is added to the filtrate.

The precipitated crystals are filtered off, washed with hexane and dried. Yield 81%, m. p. 89–90°*C*, $[a]_D^{20} = 21.6$ (C 1.000 CH_2Cl_2).

^{1}H NMR (400 *MHz*, $CDCl_3$), δ, *ppm*: 7.46 s (1H, NCH), 6.66 ddd (*J*=5.7, 2.6, 1.4 *Hz*, 1H, HC=CCO), 5.11 s (1H^{a}, H_2C=), 5.05 s (1H^{b}, H_2C=), 5.02 d (*J*=15.6 *Hz*, 1H^{a}, CH_2=C–$\underline{CH_2}$N), 4.92 d (*J*=15.2 *Hz*, 1H^{b}, CH_2=C–$\underline{CH_2}$N), 3.79 s (2H, $\underline{CH_2}N(CH_2)_6$), 2.68–2.60 m (4H, $N(\underline{CH_2}CH_2CH_2)_2$), 2.60–2.29 m (4H, CH and CH_2 in carbocycle), 2.29–2.04 m (1H, CH_2 in carbocycle), 1.74 dt (*J*=2.4, 1.2 *Hz*, 3H, CH_3), 1.68–1.51 m (8H, $N(CH_2\underline{CH_2}\underline{CH_2})_2$).

^{13}C NMR (101 *MHz*, $CDCl_3$), δ, *ppm*: 198.6 (C=O); 146.9; 145.3; 143.8; 135.8; 122.4; 115.4; 55.6; 54.0; 53.6; 42.8; 38.3; 31.3; 28.1; 27.0; 15.7.

HRMS (ESI) *m/z*: $[M+H]^+$ Calcd for $C_{19}H_{29}N_4O^+$ 329.2341. Found 329.2339.

BSA was purchased from the "Sigma Chemical Co" (USA) and was used without further purification. The concentration of BSA (0.4 *mg/mL*) was determined spectrophotometrically using a molar extinction coefficient 39080 $M^{-1}\cdot cm^{-1}$ at 280 *nm* (due to the presence of Trp, Tyr and Phe). The concentration of 1,2,3-triazolyl-carvone was varied in the range of $4.00\cdot10^{-5}$–$2.00\cdot10^{-4}$ *M*. The protein solutions were prepared in saline solution. The fluorescence spectra were recorded on a Cary Eclipse (Varian) spectrofluorometer at 298 and 308 *K*, in the range λ=300–500 *nm*

at the excitation wavelength λ=280 *nm*. The temperature of the samples was maintained constant by the water circulating using LAUDA Alpha 100 thermostat (Germany). l=1 *cm* cuvettes were used. The graphs were constructed and analyzed using the ORIGIN 8.0 software.

Determination of the thermodynamic parameters of 1,2,3-triazolyl-carvone binding to BSA. The character of binding interactions between the protein and the ligand was determined by the relationship of thermodynamic parameters (ΔH, ΔS and ΔG). The thermodynamic parameters of 1,2,3-triazolyl-carvone binding with BSA are determined using the following equations:

$$\ln K_b = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}, \quad (1)$$

$$\Delta G = \Delta H - T\Delta S, \quad (2)$$

where K_b is the binding constant and R is the gas constant. K_b and the number of binding sites (n) were determined according to the well-known Eq. (3) [14]:

$$\lg[(F_0 - F)/F] = \lg K_b + n \lg[Q], \quad (3)$$

where F_0 and F are the fluorescence intensities of BSA in the absence and presence of 1,2,3-triazolyl-carvone respectively; K_b is the binding constant; n is the number of binding sites; [Q] is the concentration of quencher 1,2,3-triazolyl-carvone. K_b and n are determined from the dependence lg(F_0–F)/F vs lg[Q].

Determination of the Distance between the Protein and Ligand. The distance between the protein and the ligand can be calculated using the theory of fluorescence resonance energy transfer (Förster's theory) [14]. The efficiency of energy transfer E is calculated using the equation

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6}, \quad (4)$$

where E is the energy transfer efficiency, F_0 and F are the fluorescence intensities of BSA in the absence and presence of 1,2,3-triazolyl-carvone respectively; r is the distance between the donor and the acceptor; R_0 is the Förster's radius, when the energy transfer efficiency is 50% and can be calculated by the following equation:

$$R_0^6 = 8.79 \cdot 10^{-25} \kappa^2 n^{-4} \Phi J, \quad (5)$$

where κ^2 is the spatial orientation factor (κ^2=2/3); n is the refractive index (1.336); Φ is the fluorescence quantum yield of the donor; J is the overlap integral of the donor fluorescence spectrum and the acceptor absorption spectrum:

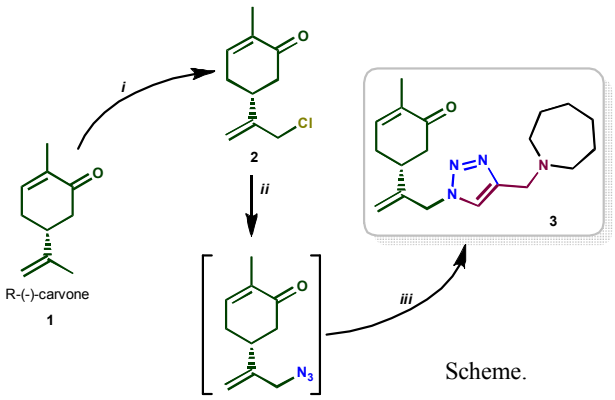
$$J = \sum F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda) / \sum F(\lambda)\Delta\lambda, \quad (6)$$

where $F(\lambda)$ is the fluorescence intensity of the donor at wavelength λ; ε (λ) is the molar extinction coefficient of the acceptor at wavelength λ. Using equations (4)–(6), the overlap integral, the energy transfer efficiency, the Förster's radius and the distance between the donor and the acceptor were calculated and given in Tab. 2.

Results and Discussion.

Synthesis of 1,2,3-triazolyl-carvone. The synthesis of (5*R*)-2-methyl-5-(3--{4-[(azepan-1-yl)methyl]-1*H*-1,2,3-triazol-1-yl}prop-1-en-2-yl)cyclohex-2-en-1-one was performed due to the Scheme. L-carvone–((*R*)-2-methyl-5-(prop-1-en-2--yl)cyclohex-2-enone) (**1**) was used as the starting raw material, which under the

action of calcium hypochlorite was converted to (*R*)-5-(chloroprop-1-en-2-yl)-2--methyl-cyclohex-2-enone (**2**). Further, the interaction of **2** with sodium azide resulted in L-carvoneazide-(*R*)-5-(azidoprop-1-en-2-yl)-2-methylcyclohex-2-enone, which without isolation under the reaction conditions of 1,3-cycloaddition upon interaction with N-propargylazepane led to the target 1,2,3-triazolyl-carvone–(5*R*)-2-methyl-5-(3-{4-[(azepan-1-yl)methyl]-1*H*-1,2,3-triazol-1-yl}prop-1-en-2-yl) cyclohex-2-en-1-one (**3**).



Scheme.

Synthesis of 1,2,3-triazolyl-carvone.

i – $Ca(OCl)_2$, CO_2, CH_2Cl_2/H_2O, 0°*C*;
ii – NaN_3 (1.2 eq.), MeCN;
iii – Et_3N (1.2 eq.), CuI (5 *mol*%), N-propargylazepane (1.2 eq.), MeCN, 60°*C*.

The synthesized compound was characterized by physicochemical constants, and the structure of **3** was identified by ^1H and ^{13}C NMR spectroscopy and HRMS analysis as well.

Fluorescence Spectroscopy Studies. BSA fluorescence quenching data by 1,2,3-triazolyl-carvone are presented in the Tab. 1.

Table 1

BSA fluorescence intensities in the presence of 1,2,3-triazolyl-carvone

Concentration of 1,2,3-triazolyl-carvone	Fluorescence intensity, a.u.	
	298 *K*	308 *K*
0	662.4	556.6
$4.0 \cdot 10^{-5}$	598.5	494.4
$6.0 \cdot 10^{-5}$	580.8	466.9
$8.0 \cdot 10^{-5}$	573.2	434.5
$1.0 \cdot 10^{-4}$	534.3	419.6
$1.2 \cdot 10^{-4}$	522.0	389.7
$1.4 \cdot 10^{-4}$	494.4	357.9
$1.6 \cdot 10^{-4}$	463.7	349.8
$2.0 \cdot 10^{-4}$	448.4	342.3

Binding Studies of 1,2,3-triazolyl-carvone with Serum Albumin. The transport of drugs is known to be carried out by various interactions with the proteins. The most abundant protein in the blood plasma is serum albumin (up to 60%), which reversibly can bind various endogenous and exogenous compounds, antibiotics, antioxidants, hormones, warfarin and others [15, 16]. In the protein molecule different specific binding sites are responsible for the transport of compounds of different structural classes. Some binding reactions occur due to the formation of

hydrogen bonds, electrostatic, hydrophobic interactions, others – provoking chemical modifications in the side radicals of amino acid moieties, are covalent in nature.

Spectroscopic methods such as absorption (electronic absorption, IR) and emission (fluorescence) are often used to study the interactions between biomolecules and ligands. Fluorescence spectroscopy has been used to study the interaction between 1,2,3-triazolyl-carvone with the transport protein bovine serum albumin (BSA).

The thermodynamic parameters (ΔH, ΔS and ΔG) are main evidences used to propose the binding mode. For the typical hydrophobic interactions, both ΔH and ΔS are positive, while negative ΔH and ΔS result from the hydrogen bond formation and van der Waals forces, and in the case when $\Delta H<0$ and $\Delta S>0$, then electrostatic interactions [17]. The value of K_b for transport protein-ligand interaction in the range of 10^3–10^6 M^{-1} indicates the reversibility of ligand binding [18]. The results obtained for BSA–1,2,3-triazolyl-carvone system are summarized in Tab. 2.

Table 2

The values of thermodynamic parameters for BSA binding with 1,2,3-triazolyl-carvone at 298 and 308 K

T, K	K_b, M^{-1}	n	ΔH, $kJ{\cdot}mol^{-1}$	ΔG, $kJ{\cdot}mol^{-1}$	ΔS, $J{\cdot}mol^{-1}K^{-1}$
298	$2.40 \cdot 10^3$	1.001 ± 0.079	67.989	–19.283	292.86
308	$5.85 \cdot 10^3$	1.061 ± 0.066		–22.212	

Negative values of ΔG show that the binding process proceeds spontaneously. The positive values of ΔH and ΔS show that the stability of the BSA–1,2,3-triazolyl-carvone system is mainly specified by hydrophobic interactions.

The distance between the protein and the ligand can be calculated using the theory of resonance energy transfer (Förster's theory). The results obtained for the BSA–1,2,3-triazolyl-carvone system are given in Tab. 3.

Table 3

The values of the overlap integral, the energy transfer efficiency, the Förster radius and the distance between the BSA and 1,2,3-triazolyl-carvone at 298 and 308K

T, K	I, $cm^3{\cdot}L{\cdot}mol^{-1}$	E	R_0, nm	r, nm
298	$1.79 \cdot 10^{-14}$	0.019	3.778	7.27
308	$1.80 \cdot 10^{-14}$	0.043	3.782	7.06

The results obtained, show that with an increase of the temperature the distance between BSA–1,2,3-triazolyl-carvone decreases. However, the fact that the intensity of interactions increases with the rise of temperature, the interactions of BSA with 1,2,3-triazolyl-carvone remain weak.

Conclusion. In this paper the synthesis, structure and binding affinity of 1,2,3-triazolyl-carvone with BSA has been studied. The thermodynamic parameters ΔH, ΔG and ΔS for this system were calculated. The positive values of enthalpy change and entropy change indicate that hydrophobic interactions played major roles in stabilizing the complex. Using Förster's energy transfer theory the distance

r between the donor (BSA) and acceptor has been calculated to be 7.27 and 7.07 *nm* at 298 and 308 *K* respectively.

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