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Article

Synthesis, Properties and Spatial Structure of 4-[(3,5-dimethyl-1,2-oxazol-4-yl)sulfonyl]cytisine

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Abstract: This article has been studied the synthesis of a new derivative of the known alkaloid cytisine contained in the seeds of plants of Cytisus laburnum L. and Thermopsis lanceolata R.Br., both of the Lugiminosae family. The new compound has been obtained from two biologically active compounds such as isoxazole and cytisine. It has been demonstrated that the reaction led to the single-stage method under very mild conditions to obtain some 4-[(3,5-dimethyl-1,2-oxazole-4-yl)sulfonyl]amides. This class of compounds is promising to obtain the new biologically active compounds. This article has examined in detail a structure with using the 1H and 13C NMR and two-dimensional NMR spectroscopy of COSY (1H-1H), HMQC (1H-13C) and NMVS (1H-13C). As a result, the homo- and heteronuclear spin-spin couplings should be established. The X-ray diffraction analysis has determined the spatial structure of a new derivative based on cytisine alkaloid. Thus, its hemorheological activity has been studied.

Keywords: sulfo-derivatives of azoles; alkaloid cytisine; spatial structure; hemorheological activity; ^{1}H; ^{13}C; 2D NMR spectroscopy; X-ray diffraction analysis

1. Introduction

The sulfo-derivatives of azoles have been widely used in pharmacy as biologically active compounds [1]. Noteworthy among them is a group of the nonsteroidal anti-inflammatory drugs such as coxibs. They inhibit cyclooxygenase [2]. The study of the biological activity of azole-containing systems has included a group of sulfonamides. Their representatives had some sulfonamide derivatives of phenylisoxazole such as sulfamethoxazole [3], sulfafurazole [4], sulfamoxole [5] and sulfafenazole used in the veterinary medicine [6]. Sulfonamides are antibacterial agents that inhibit the growth of the gram-negative and gram-positive bacteria.

Addition of a sulfonamide fragment into a natural compound's molecule, in particular an alkaloid cytisine, is of particular interest, i.e. the obtained compounds inhibit human carbonic anhydrase. Study of the carbonic anhydrase inhibitors is a rapidly developing area in the medicinal chemistry. Thus, the carbonic anhydrase plays a key role in some biochemical processes based on physiology of the pathological states [7, 8].

2. Results and Discussion

This study has used 3,5-dimethylisoxazole with a wide spectrum of its biological activity. Some publications have described a physiological activity of compounds derived from sulfo-derivatives of 3,5-dimethylisoxazole [9-12]. The published results have demonstrated that to develop the synthesis methods for the new isoxazole derivatives was an urgent task in the medicinal chemistry. Therefore, modification of a natural alkaloid

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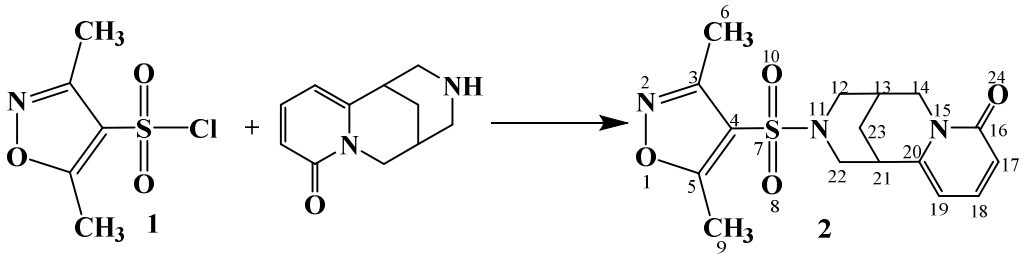
cytisine with adding an isoxazole molecule (or a substituted isoxazole) of sulfogroup is one of the advancing directions to search the new biologically active compounds.

The 3,5-dimethylisoxazole has been sulfochlorinated as described in [13]. Two methyl groups in a 3,5-dimethylisoxazole molecule have increased the electron density on the ring. Thus, it has intensified the probability of sulfochlorination at position 4.

The 4-[(3,5-dimethyl-1,2-oxazol-4-yl)sulfonyl]cytisine **2** has been obtained by reacting of a sulfochloride **1** with cytisine in the presence of a pyridine base.



Sulfamide **2** has been synthesized by reaction of sulfochloride **1** with cytisine in dry acetonitrile in the presence of pyridine as an acid-binding agent. The structure of 4-[(3,5-dimethyl-1,2-oxazol-4-yl)sulfonyl]cytisine **2** has been proved with ^{1}H NMR spectroscopy.

The method has been used because the studied sulfamide **2** had some diverse and characteristic protons and their signals could be easy to identify in ^{1}H NMR spectrum.

Thus, ^{1}H NMR spectrum of compound **2** had the protons of the bispidine cycles of cytisine fragment at 1.76-1.85 m (2H, H-23ax, 23eq), 2.49 s (4H, H-9,9,9,13), 2.83 d (1H, H-12ax, ^{2}J 11.4 Hz), 2.92 d (1H, H -12eq, ^{2}J 11.4 Hz), 3.22 s (1H, H-21), 3.56 d (1H, H-22ax, ^{2}J 10.8 Hz), 3.61 d (1H, H-22eq, ^{2}J 10.8 Hz), and 3.73-3.83 m (2H, H-14ax,14eq) ppm. The aromatic protons of a cytisine group have been recorded at 6.30 br. s (1H, H-17), 6.36 d (1H, H-19, ^{3}J 9.2 Hz), and 7.43 t (1H, H-18, ^{2}J 6.6 Hz) ppm. The methyl protons of H-6,6,6 and H-9,9,9 of an isoxazole fragment have been observed as triplet singlets at 2.12 ppm and 2.49 ppm, respectively.

The ^{13}C NMR spectrum of compound **2** has demonstrated the signals of carbon atoms of a cytisine fragment at 24.07 (C-23), 26.59 (C-13), 33.60 (C-21), 49.70 (C-14), 51.20 (C-22), C-12), 106.91 (C-19), 115.90 (C-17), 140.37 (C-18), 150.60 (C-20) and 162.45 (C-16) ppm. The carbon atoms of a dimethylisoxazole fragment have been recorded at 11.04 (C-6), 12.95 (C-9), 113.30 (C-4), 158.21 (C-3) and 174.45 (C-5) ppm.

A structure of compound **2** has been confirmed with 2D NMR spectroscopy of COZY (^{1}H-^{1}H), HMQC (^{1}H-^{13}C) and HMBC (^{1}H-^{13}C). Thus, the homo- and hetero-nuclear spin-spin coupling has been established.

The observed NMR correlations of COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) in a molecule are illustrated in Figure 1.

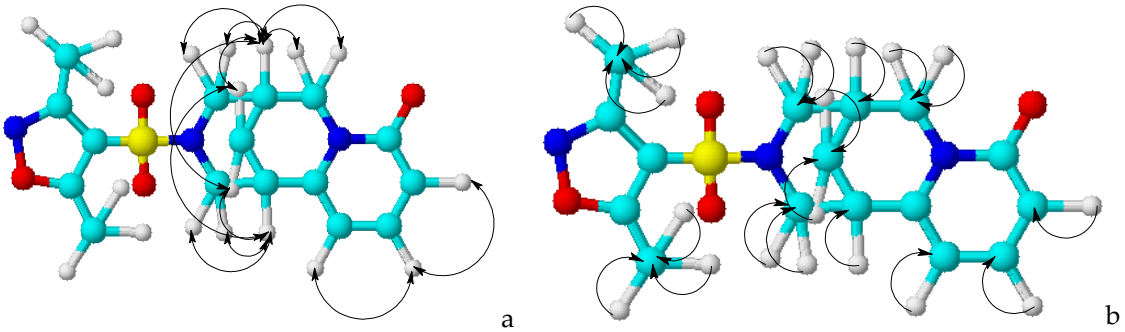


Figure 1. The structural correlations in COSY (a) and HMQC (b) spectra of compound **2.**

The ^{1}H-^{1}H COZY spectra of compound **2** have demonstrated the spin-spin correlations through three proton bonds of near methylene-methylene, methine-methylene and methine-methine groups of H^{23}-H^{21} (1.80, 3.21 and 3.21, 1.80), H^{13}-H^{14} (2.50, 3.76 and 3.76,

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2.50), H^{12ax}-H^{22eq} (2.81, 3.60 and 3.60, 2.81), H^{12eq}-H^{22ax} (2.90, 3.49 and 3.49, 3.49), H^{19}-H^{18} (6.28, 7.42 and 7.42. 6.28) and H^{17}-H^{18} (6.31, 7.41 and 7.41, 6.31) ppm.

Hetero-nuclear couplings of protons with carbon atoms through a single bond have been established by ^{1}H-^{13}C HMQC spectroscopy for the following pairs in a compound: H^{6}-C^{6} (2.10, 11.37), H^{9}-C^{9} (2.47, 12.85), H^{23}-C^{23} (1.79, 24.74), H^{13}-C^{13} (2.50, 26.87), H^{21}-C^{21} (3.20, 33.83), H^{12ax}-C^{12} (2.81, 52.89), H^{12eq}-C^{12} (2.93, 52.44), H^{22ax}-C^{22} (3.46, 51.14),H^{22eq}-C^{22} (3.60, 51.38), H^{14}-C^{14} (3.76, 50.01), H^{19}-C^{19} (6.28, 107.35), H^{17}-C^{17} (6.34, 116.27) and H^{18}-C^{18} (7.42, 140.69) ppm.

Hetero-nuclear couplings of protons with carbon atoms through two or more bonds have been determined by ^{1}H-^{13}C HMBC spectroscopy for the following pairs in a compound: H^{19}-C^{17} (6.28, 116.38), H^{19}-C^{20} (6.28, 150.54); H^{9}-C^{4} (2.47, 114.10), H^{9}-C^{3} (2.47, 157.09), H^{9}-C^{5} (2.47, 175.02); H^{6}-C^{4} (2.10, 114.11), H^{6}-C^{3} (2.10, 158.22) and H^{23}-C^{20} (1.78, 150.54) ppm.

In order to establish a spatial structure of the obtained 4-[(3,5-dimethyl-1,2-oxazol-4-yl)sulfonyl]cytisin **2**, its X-ray diffraction analysis has been performed. A general view of a molecule is illustrated in Figure 2.

An absolute configuration has been defined reliably. Configuration of the C7 and C9 chiral centers has been correlated with the known quantity in the (-)-cytisine molecule [14]. Flack parameter has been 0.01(9) [15]. A five-membered cycle has been disordered (180° rotation around the C-S bond) in the ratio of 0.72:0.78.

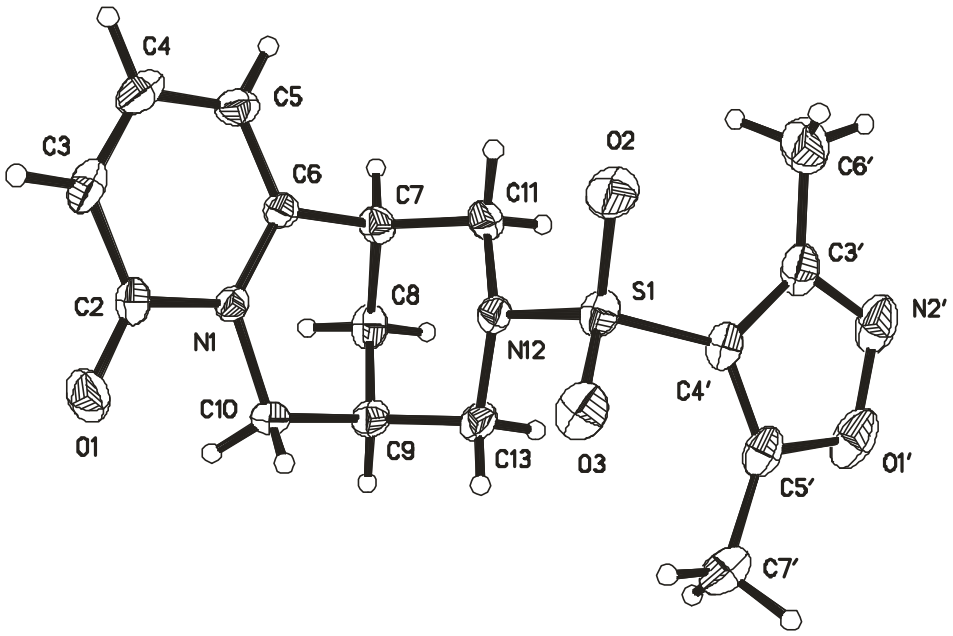

Figure 2. A general view of molecule **2.**

The bond lengths (Table 1) and bond angles (Table 2) in a cytisine skeleton of structure **2** have been found to be standard [16], except for bond angles of N12 atom (Table 2).

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Table 1. Bond lengths (d, Å) in molecule **2.**

Bond	d	Bond	d
S(1)-O(3)	1.424(2)	C(9)-C(13)	1.523(4)
S(1)-O(2)	1.427(2)	C(9)-H(9A)	0.9800
S(1)-N(12)	1.634(2)	C(10)-H(10A)	0.9700
S(1)-C(4')	1.749(3)	C(10)-H(10B)	0.9700
O(1)-C(2)	1.231(3)	C(11)-N(12)	1.472(4)
N(1)-C(6)	1.372(3)	C(11)-H(11A)	0.9700
N(1)-C(2)	1.400(3)	C(11)-H(11B)	0.9700
N(1)-C(10)	1.487(3)	N(12)-C(13)	1.484(3)
C(2)-C(3)	1.427(4)	C(13)-H(13A)	0.9700
C(3)-C(4)	1.348(5)	C(13)-H(13B)	0.9700
C(3)-H(3B)	0.9300	O(1')-C(5')	1.334(4)
C(4)-C(5)	1.396(4)	O(1')-N(2')	1.418(4)
C(4)-H(4A)	0.9300	N(2')-C(3')	1.304(4)
C(5)-C(6)	1.362(4)	C(3')-C(4')	1.412(4)
C(5)-H(5A)	0.9300	C(3')-C(6')	1.475(6)
C(6)-C(7)	1.500(4)	C(4')-C(5')	1.372(4)
C(7)-C(8)	1.526(4)	C(5')-C(7')	1.480(5)
C(7)-C(11)	1.532(4)	C(6')-H(6'A)	0.9600
C(7)-H(7A)	0.9800	C(6')-H(6'B)	0.9600
C(8)-C(9)	1.515(5)	C(6')-H(6'C)	0.9600
C(8)-H(8A)	0.9700	C(7')-H(7'A)	0.9600
C(8)-H(8B)	0.9700	C(7')-H(7'B)	0.9600
C(9)-C(10)	1.512(4)	C(7')-H(7'C)	0.9600

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Table 2. Bond angles (ω, deg.) in molecule **2.**

Angle	ω	Angle	ω
O(3)-S(1)-O(2)	120.01(14)	N(1)-C(10)-H(10A)	108.6
O(3)-S(1)-N(12)	107.74(13)	C(9)-C(10)-H(10A)	108.6
O(2)-S(1)-N(12)	107.29(13)	N(1)-C(10)-H(10B)	108.6
O(3)-S(1)-C(4')	106.65(14)	C(9)-C(10)-H(10B)	108.6
O(2)-S(1)-C(4')	107.25(14)	H(10A)-C(10)-H(10B)	107.6
N(12)-S(1)-C(4')	107.31(12)	N(12)-C(11)-C(7)	108.9(2)
C(6)-N(1)-C(2)	123.0(2)	N(12)-C(11)-H(11A)	109.9
C(6)-N(1)-C(10)	123.5(2)	C(7)-C(11)-H(11A)	109.9
C(2)-N(1)-C(10)	113.4(2)	N(12)-C(11)-H(11B)	109.9
O(1)-C(2)-N(1)	119.6(3)	C(7)-C(11)-H(11B)	109.9
O(1)-C(2)-C(3)	124.8(3)	H(11A)-C(11)-H(11B)	108.3
N(1)-C(2)-C(3)	115.6(3)	C(11)-N(12)-C(13)	113.4(2)
C(4)-C(3)-C(2)	121.2(3)	C(11)-N(12)-S(1)	117.54(17)
C(4)-C(3)-H(3B)	119.4	C(13)-N(12)-S(1)	115.02(19)
C(2)-C(3)-H(3B)	119.4	N(12)-C(13)-C(9)	109.5(2)
C(3)-C(4)-C(5)	120.8(3)	N(12)-C(13)-H(13A)	109.8
C(3)-C(4)-H(4A)	119.6	C(9)-C(13)-H(13A)	109.8
C(5)-C(4)-H(4A)	119.6	N(12)-C(13)-H(13B)	109.8
C(6)-C(5)-C(4)	120.1(3)	C(9)-C(13)-H(13B)	109.8
C(6)-C(5)-H(5A)	120.0	H(13A)-C(13)-H(13B)	108.2
C(4)-C(5)-H(5A)	120.0	C(5')-O(1')-N(2')	108.7(2)
C(5)-C(6)-N(1)	119.3(2)	C(3')-N(2')-O(1')	106.7(3)
C(5)-C(6)-C(7)	122.0(2)	N(2')-C(3')-C(4')	110.1(3)
N(1)-C(6)-C(7)	118.7(2)	N(2')-C(3')-C(6')	118.5(3)
C(6)-C(7)-C(8)	110.9(2)	C(4')-C(3')-C(6')	131.4(3)
C(6)-C(7)-C(11)	111.1(2)	C(5')-C(4')-C(3')	105.9(3)
C(8)-C(7)-C(11)	109.8(2)	C(5')-C(4')-S(1)	126.7(2)
C(6)-C(7)-H(7A)	108.3	C(3')-C(4')-S(1)	127.4(2)
C(8)-C(7)-H(7A)	108.3	O(1')-C(5')-C(4')	108.6(3)
C(11)-C(7)-H(7A)	108.3	O(1')-C(5')-C(7')	117.0(3)
C(9)-C(8)-C(7)	106.5(2)	C(4')-C(5')-C(7')	134.4(3)
C(9)-C(8)-H(8A)	110.4	C(3')-C(6')-H(6'A)	109.5
C(7)-C(8)-H(8A)	110.4	C(3')-C(6')-H(6'B)	109.5
C(9)-C(8)-H(8B)	110.4	H(6'A)-C(6')-H(6'B)	109.5
C(7)-C(8)-H(8B)	110.4	C(3')-C(6')-H(6'C)	109.5
H(8A)-C(8)-H(8B)	108.6	H(6'A)-C(6')-H(6'C)	109.5
C(10)-C(9)-C(8)	111.1(2)	H(6'B)-C(6')-H(6'C)	109.5
C(10)-C(9)-C(13)	112.2(2)	C(5')-C(7')-H(7'A)	109.5
C(8)-C(9)-C(13)	110.4(2)	C(5')-C(7')-H(7'B)	109.5
C(10)-C(9)-H(9A)	107.6	H(7'A)-C(7')-H(7'B)	109.5
C(8)-C(9)-H(9A)	107.6	C(5')-C(7')-H(7'C)	109.5
C(13)-C(9)-H(9A)	107.6	H(7'A)-C(7')-H(7'C)	109.5
N(1)-C(10)-C(9)	114.4(2)	H(7'B)-C(7')-H(7'C)	109.5

A dihydropyridine cycle has been flat at ± 0.007 Å. A carbonyl O1 atom almost has been in plane of atoms of this cycle. Deviation has been 0.03 Å.

The N1C6C7C8C9C10 tetrahydropyridine cycle has been in a distorted 8α-chair conformation (ΔC_s^8=3.02°). A bridging C8 atom has been observed from a middle plane of

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the remaining atoms (±0.01 Å) of the cycle by 0.73 Å. A piperidine cycle has been in a distorted chair conformation (ΔC_S^8=0.99°, $\Delta C_2^{7,8}$=1.67°). An isoxazole cycle has been planar and its accuracy -± 0.002 Å. The methyl groups have been found in a plane of the five-membered cycle. Values of torsion angles are presented in Table 3.

Table 3. Torsion angles (τ, deg.) in molecule **2.**

Angle	τ	Angle	τ
C(6)-N(1)-C(2)-O(1)	179.4(2)	O(3)-S(1)-N(12)-C(11)	168.7(2)
C(10)-N(1)-C(2)-O(1)	1.6(4)	O(2)-S(1)-N(12)-C(11)	38.1(2)
C(6)-N(1)-C(2)-C(3)	-0.9(4)	C(4')-S(1)-N(12)-C(11)	-76.8(2)
C(10)-N(1)-C(2)-C(3)	-178.7(2)	O(3)-S(1)-N(12)-C(13)	-53.9(2)
O(1)-C(2)-C(3)-C(4)	-178.3(3)	O(2)-S(1)-N(12)-C(13)	175.6(2)
N(1)-C(2)-C(3)-C(4)	2.0(4)	C(4')-S(1)-N(12)-C(13)	60.6(2)
C(2)-C(3)-C(4)-C(5)	-1.2(5)	C(11)-N(12)-C(13)-C(9)	-56.2(3)
C(3)-C(4)-C(5)-C(6)	-0.9(5)	S(1)-N(12)-C(13)-C(9)	164.5(2)
C(4)-C(5)-C(6)-N(1)	1.9(4)	C(10)-C(9)-C(13)-N(12)	-66.1(3)
C(4)-C(5)-C(6)-C(7)	-177.7(3)	C(8)-C(9)-C(13)-N(12)	58.4(3)
C(2)-N(1)-C(6)-C(5)	-1.1(4)	C(5')-O(1')-N(2')-C(3')	-0.2(4)
C(10)-N(1)-C(6)-C(5)	176.5(3)	O(1')-N(2')-C(3')-C(4')	0.4(4)
C(2)-N(1)-C(6)-C(7)	178.6(2)	O(1')-N(2')-C(3')-C(6')	178.5(4)
C(10)-N(1)-C(6)-C(7)	-3.8(3)	N(2')-C(3')-C(4')-C(5')	-0.4(4)
C(5)-C(6)-C(7)-C(8)	-148.2(3)	C(6')-C(3')-C(4')-C(5')	-178.2(4)
N(1)-C(6)-C(7)-C(8)	32.1(3)	N(2')-C(3')-C(4')-S(1)	178.4(2)
C(5)-C(6)-C(7)-C(11)	89.3(3)	C(6')-C(3')-C(4')-S(1)	0.6(6)
N(1)-C(6)-C(7)-C(11)	-90.3(3)	O(3)-S(1)-C(4')-C(5')	29.7(3)
C(6)-C(7)-C(8)-C(9)	-60.9(3)	O(2)-S(1)-C(4')-C(5')	159.4(3)
C(11)-C(7)-C(8)-C(9)	62.3(3)	N(12)-S(1)-C(4')-C(5')	-85.6(3)
C(7)-C(8)-C(9)-C(10)	63.5(3)	O(3)-S(1)-C(4')-C(3')	-148.9(3)
C(7)-C(8)-C(9)-C(13)	-61.6(3)	O(2)-S(1)-C(4')-C(3')	-19.1(3)
C(6)-N(1)-C(10)-C(9)	5.7(4)	N(12)-S(1)-C(4')-C(3')	95.9(3)
C(2)-N(1)-C(10)-C(9)	-176.5(2)	N(2')-O(1')-C(5')-C(4')	0.0(3)
C(8)-C(9)-C(10)-N(1)	-36.3(3)	N(2')-O(1')-C(5')-C(7')	-179.3(3)
C(13)-C(9)-C(10)-N(1)	87.8(3)	C(3')-C(4')-C(5')-O(1')	0.3(3)
C(6)-C(7)-C(11)-N(12)	63.2(3)	S(1)-C(4')-C(5')-O(1')	-178.6(2)
C(8)-C(7)-C(11)-N(12)	-59.9(3)	C(3')-C(4')-C(5')-C(7')	179.3(4)
C(7)-C(11)-N(12)-C(13)	56.9(3)	S(1)-C(4')-C(5')-C(7')	0.5(5)
C(7)-C(11)-N(12)-S(1)	-164.97(18)		

Configuration of nitrogen atom (N12) in a pyridine cycle of molecule **2** has been observed to be intermediate between pyramidal and trigonal planar. The sum of bond angles has been 345.9°. It has been caused by a partial interaction between a lone electron pair of nitrogen atom N12 and π-electrons of S=O bonds. A pyramidal configuration of nitrogen atom has been found in the N-derivatives of cytosine. As a result, the addition of any groups has not lead to conjugation of nitrogen lone electron pair with electrons of these groups. Thus, the electronic configuration of N12 atom has been pyramidal in molecules of nitromethylcytisine [17] and cytisine [18] without the corresponding conjugation. The sum of bond angles has been 333.7 and 338.3°, respectively. Here and below the data on the crystal structures have been taken from the Cambridge Structural Database [19].

Various steric strain groups have been found in cytisine derivatives conjugated with a nitrogen lone electron pair. Thus, atom configuration has changed, and it had a trigonal

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planar form e.g. in molecules of ammonium N-cytisine dithiocarbamate crystal hydrate [20] and N-formylcytisine [21]. It has been explained with a mesomeric effect with conjugation between the nitrogen lone electron pair and the S-C-S (C=O) bond. Table 4 demonstrates the atomic coordinates of a structure in cell fractions of compound **2**.

Table 4. The atomic coordinates (x10^4) of structure in cell fraction **1.**

Atom	x	y	z
S(1)	1570(1)	6334(1)	2084(1)
O(1)	3784(4)	4085(3)	6024(2)
O(2)	-479(3)	5822(3)	1848(2)
O(3)	2015(4)	7585(3)	2713(2)
N(1)	3553(3)	3144(2)	4497(2)
C(2)	2863(4)	3270(3)	5396(2)
C(3)	1088(5)	2419(4)	5507(2)
C(4)	195(5)	1522(4)	4792(3)
C(5)	965(4)	1421(4)	3914(2)
C(6)	2621(4)	2250(3)	3765(2)
C(7)	3456(4)	2222(3)	2821(2)
C(8)	5760(4)	2453(4)	2978(2)
C(9)	6137(4)	3981(4)	3445(2)
C(10)	5433(4)	4025(4)	4428(2)
C(11)	2442(4)	3421(3)	2127(2)
N(12)	2927(3)	4914(3)	2551(2)
C(13)	5147(4)	5205(4)	2775(2)
O(1')	4206(5)	7819(3)	-55(2)
N(2')	2656(6)	6928(3)	-579(2)
C(3')	1633(5)	6326(4)	55(2)
C(4')	2446(4)	6796(3)	997(2)
C(5')	4050(5)	7722(3)	885(2)
C(6')	-125(9)	5360(5)	-294(3)
C(7')	5530(5)	8596(4)	1557(3)

To synthesize the sulfonamide derivatives of 3-methyl-5-vinyl-substituted isoxazoles, a method has been developed [22]. It has been based on preliminary functionalization of the 3,5-dimethylisoxazole molecule. The reaction of sulfochlorination and amination with cytosine has been performed. As a result, a methyl group has been activated at 5-position and a vinyl fragment has been constructed. The 4-[(3,5-dimethyl-1,2-oxazol-4-yl)sulfonyl]cytisine **2** with its electron-accepting properties has demonstrated that a sulfamide group at 4-position of the isoxazole cycle has increased an acidity of methyl groups at 3- and 5-positions. In order to establish a preferential electron-accepting effect of sulfogroup on a methyl group at 3- or 5-positions, a condensation reaction has been performed with *p*-chlorobenzaldehyde. As a result, a methyl group at 5-position of an isoxazole cycle has been more active. Thus, a condensation product by a methyl group at 5-position of **3** has been obtained. A reaction has proceeded with strong bases and potassium hydroxide. The mixture has been heated for 1 h at 60°C. Regioselectivity of the reaction has been established with a combination of the NMR spectroscopy methods.

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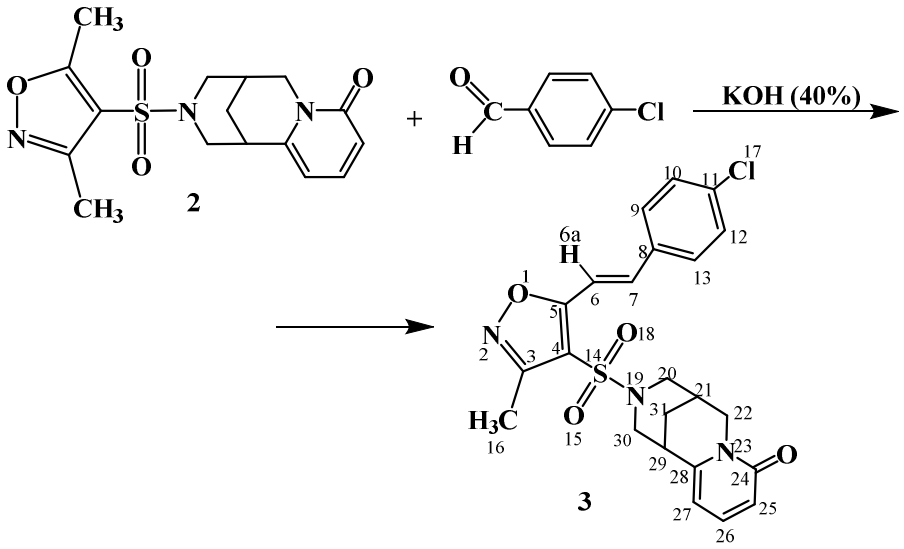

The ^{1}H NMR spectrum of compound **3** has been characterized by protons of the bispidine cycles of a cytisine fragment at 1.74-1.77 m (2H, H-31ax,31eq), 2.45 s (1H, H-21), 2.81 d (1H, H-20ax, ^{2}J 11.6 Hz), 2.91 d (1H, H-20eq, ^{2}J 11.6 Hz), 3.17 s (1H, H-29), 3.29-3.83 m (H-22ax,22eq,30ax,30eq) ppm. The aromatic protons of a cytisine group have been recorded at 6.15-6.17 m (1H, H-27), 6.22-6.24 m (1H, H-25) and 7.30-7.33 m (1H, H-26) ppm.

The methyl protons of the H-16,16,16 oxazole fragment have been observed as a three-proton singlet in a strong-field region of spectrum at 2.5 ppm. Thus, it has been at 5-position at 2.50 ppm. The aromatic protons of a chlorophenyl fragment of H-10,12 and H-9,13 has been resonated with two-proton multiplets at 7.46-7.49 and 7.69-7.71 ppm, respectively. For protons of a vinyl fragment at 3-position of an isoxazole cycle, the spectrum has been characterized with two doublet signals at 7.19 ppm (H-6) and 7.63 ppm (H-7). The high spin-spin coupling constant has been 16.5 Hz. Hence, an ethylene fragment in the form of E-diastereomer has been observed.

The ^{13}C NMR spectrum of compound **3** has demonstrated the signals of carbon atoms of a cytisine fragment at 24.12 (C-31), 26.75 (C-21), 33.87 (C-29), 49.27 (C-30), 51.07 (C-22), 52.80 (C-20), 105.65 (C-27), 117.13 (C-25), 140.08 (C-26), 150.11 (C-28) and 162.62 (C-24) ppm. The carbon atoms of a methylchlorophenyloxazole fragment have been recorded at 13.01 (C-16), 112.57 (C-4), 129.58 (C-6), 130.28 and 131.64 (C-9,10,12,13), 133.96 (C-11), 138.52 (C-7,8), 150.11 (C-5) and 158.52 (C-3) ppm.

A structure of compound **3** has been confirmed by 2D NMR spectroscopy of COZY (^{1}H-^{1}H), HMQC (^{1}H-^{13}C) and HMBC (^{1}H-^{13}C). Thus, the homo- and hetero-nuclear spin-spin coupling has been established.

The observed NMR correlations of COSY (^{1}H-^{1}H) and HMQC (^{1}H-^{13}C) in a molecule are illustrated in Figure 4.

The ^{1}H-^{1}H COZY spectra of compound **3** has demonstrated the spin-spin correlations through three proton bonds of near methylene-methylene, methine-methylene and methine-methine groups of H^{31}-H^{29} (1.76, 3.16 and 3.16, 1.76), H^{20eq}-H^{30eq} (2.90, 3.58 and 3.58, 2.90), H^{20eq}-H^{30ax} (2.88, 3.49 and 3.49, 2.88), H^{25}-H^{26} (6.21, 7.33 and 7.33, 6.21), H^{27}-H^{26} (6.16, 7.32 and 7.32, 6.16), H^{6}-H^{7} (7.20, 7.63 and 7.63, 7.20) and $H^{10,12}$-$H^{9,13}$ (7.46, 7.70 and 7.70, 7.46) ppm.

Hetero-nuclear couplings of protons with carbon atoms through a single bond have been established by ^{1}H-^{13}C HMQC spectroscopy for the following pairs in a compound: H^{31}-C^{31} (1.76, 24.48), H^{21}-C^{21} (2.8, 26.78), H^{16}-C^{16} (2.49, 13.01), H^{20ax}-C^{20} (2.78, 52.03), H^{20eq}-C^{20} (2.90, 51.61), H^{22ax}-C^{22} (3.53, 51.61), H^{22eq}-C^{22} (3.70, 51.61), H^{29}-C^{29} (3.16, 33.87), H^{30ax}-C^{30} (3.49, 49.81), H^{30eq}-C^{30} (3.60, 50.20), H^{26}-C^{26} (7.31, 140.08), H^{7}-C^{7} (7.63, 138.62), H^{6}-C^{6} (7.20, 128.82), H^{25}-C^{25} (6.22, 117.13), H^{27}-C^{27} (6.16, 105.65), $H^{10,12}$-$C^{10,12}$ (7.45, 130.07) and $H^{9,13}$-$C^{9,13}$ (7.69, 130.28) ppm.

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Hetero-nuclear couplings of protons with carbon atoms through two or more bonds have been determined by ^{1}H-^{13}C HMBC spectroscopy for the following pairs in a compound: H^{16}-C^{4} (2.49, 113.39) ppm.

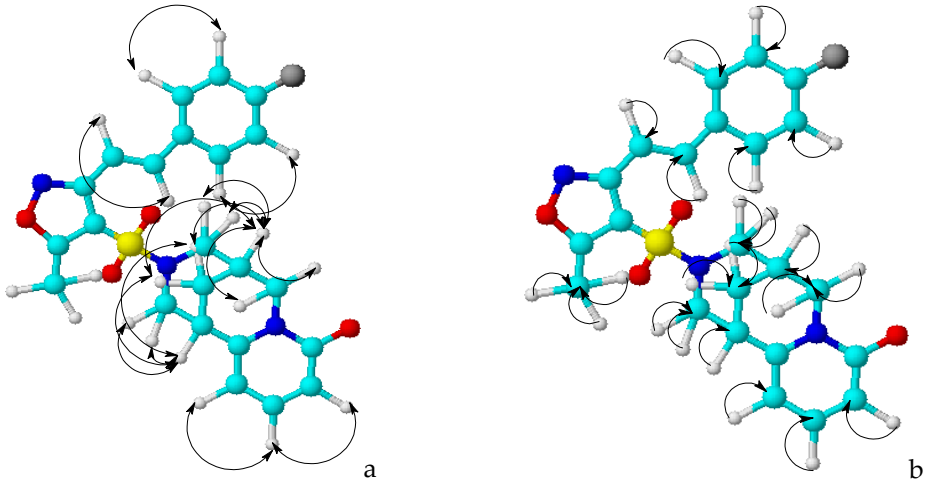

Figure 4. The structural correlations in COSY (a) and HMQC (b) spectra of compound **3.**

An *in vitro* biological screening has been performed for a hemorheological activity of compound **2**.

Hemorheological activity

The experiments on the hemorheological activity of sample **2** have been found that blood incubation for 60 min at a temperature of 43.0 °C has led to a significant increase in blood viscosity at various spindle speeds from 2 s^{-1} to 60 s^{-1}. Hence, the formation blood hyperviscosity has been observed [23].

Table 5 and Figure 4 have quoted the screening results of compound **2** for the hemorheological activity in the *in vitro* model of blood hyperviscosity.

Table 5. Effect of compound **2** on blood viscosity (mPa*s) at various spindle speeds in the *in vitro* model of blood hyperviscosity.

Tested parameter	**Blood viscosity (mPa*s) at various spindle speeds, rpm**							
	2	**4**	**6**	**8**	**12**	**20**	**40**	**60**
Reference viscosity, n=2	2.71±0.05	2.25±0.02	2.05±0.01	1.80±0.04	1.68±0.04	1.45±0.02	1.30±0.6	1.27±0.05
Blood viscosity after 1 h of incubation at 43° C in a control, n=4	8.75±0.26 p1=0.0001	7.05±0.14 p1=0.00002	5.30±0.07 p1=0.00001	4.46±0.10 p1=0.0001	3.65±0.29 p1=0.0113	3.33±0.42 p1=0.0420	3.01±0.37 p1=0.0368	2.85±0.41 p1=0.0627
Blood viscosity after 1 h of incubation at 43° C, samples with **MIN-1**, n=4	6.83±0.10 p1=0.00001 p2=0.0005	5.21±0.06 p1=0.00001 p2=0.00002	4.74±0.08 p1=0.00003 p2=0.0021	3.81±0.08 p1=0.0001 p2=0.0018	3.01±0.6 p1=0.0261 p2=0.1511	2.67±0,.9 p1=0.0118 p2=0.2044	2.32±0.15 p1=0.0109 p2=0.1332	2.17±0.10 p1=0.0040 p2=0.1580

Note:
n - number of samples in a group; p - significance level;
p1<0.05 - statistically significant differences compared to original values;
p2<0.05 - statistically significant differences compared to proper values in the control samples

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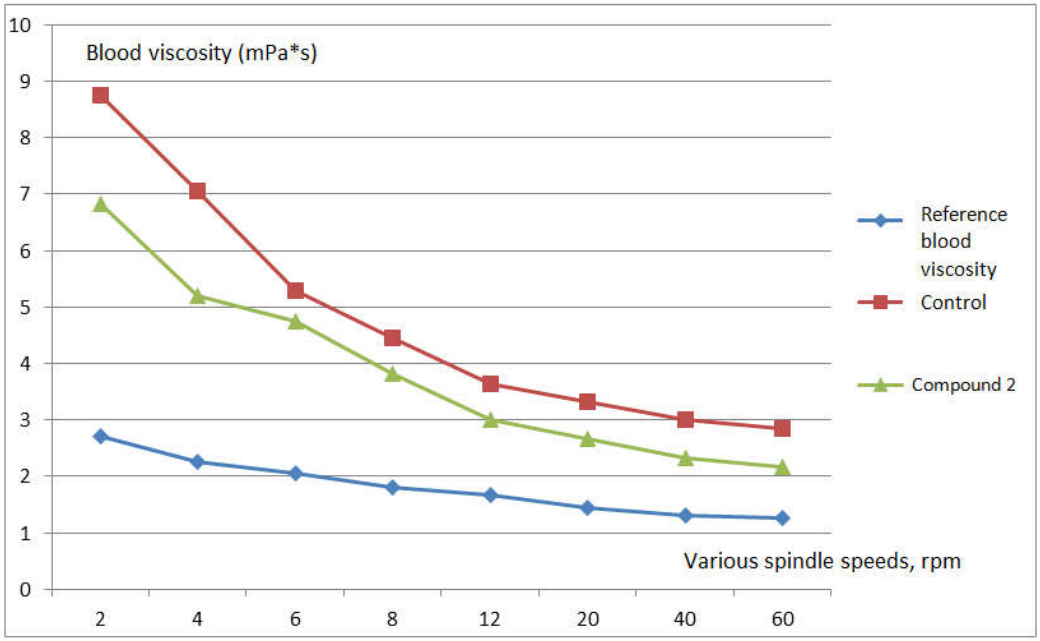

Figure 4. The hemorheological activity of compound **2.**

In order to perform a comparative analysis on a hemorheological activity, the similar experiments have been made with a reference drug such as an angioprotector pentoxifylline. Results of these experiments are listed in Table 6 and Figure 5.

Table 6. Effect of pentoxifiline on blood viscosity (mPa*s) at various spindle speeds in the *in vitro* blood hyperviscosity model.

Tested parameter	**Blood viscosity (mPa*s) at various spindle speeds, rpm**							
	2	**4**	**6**	**8**	**12**	**20**	**40**	**60**
Reference viscosity, n=2	5.94±0.59	4.90±0.43	4.10±0.38	3.87±0.34	3.40±0.29	2.69±0.26	2.32±0.12	2.21±0.12
Blood viscosity after 1 h of incubation at 43° C in a control, n=4	7.53±0.45 p1=0.0519	6.36±0.40 p1=0.0364	5.79±0.44 p1=0.0250	5.19±0.31 p1=0.0184	4.37±0.13 p1=0.0026	3.56±0.15 p1=0.0065	2.76±0.09 p1=0.0098	2.53±0.07 p1=0.0218
Blood viscosity after 1 h of incubation at 43° C, samples with pentoxifylline, n=4	7.03±0.43 p1=0.1584 p2=0.4306	5.81±0.30 p1=0.1009 p2=0.2800	5.00±0.21 p1=0.0357 p2=0.1205	4.56±0.16 p1=0.0532 p2=0.0855	4.05±0.10 p1=0.0171 p2=0.0631	3.24±0.14 p1=0.0563 p2=0.1353	2.56±0.08 p1=0.0960 p2=0.0999	2.39±0.07 p1=0.1887 p2=0.1590
Note: n - number of samples in a group; p - significance level; p1<0.05 - statistically significant differences compared to original values; p2<0.05 - statistically significant differences compared to proper values in the control samples								

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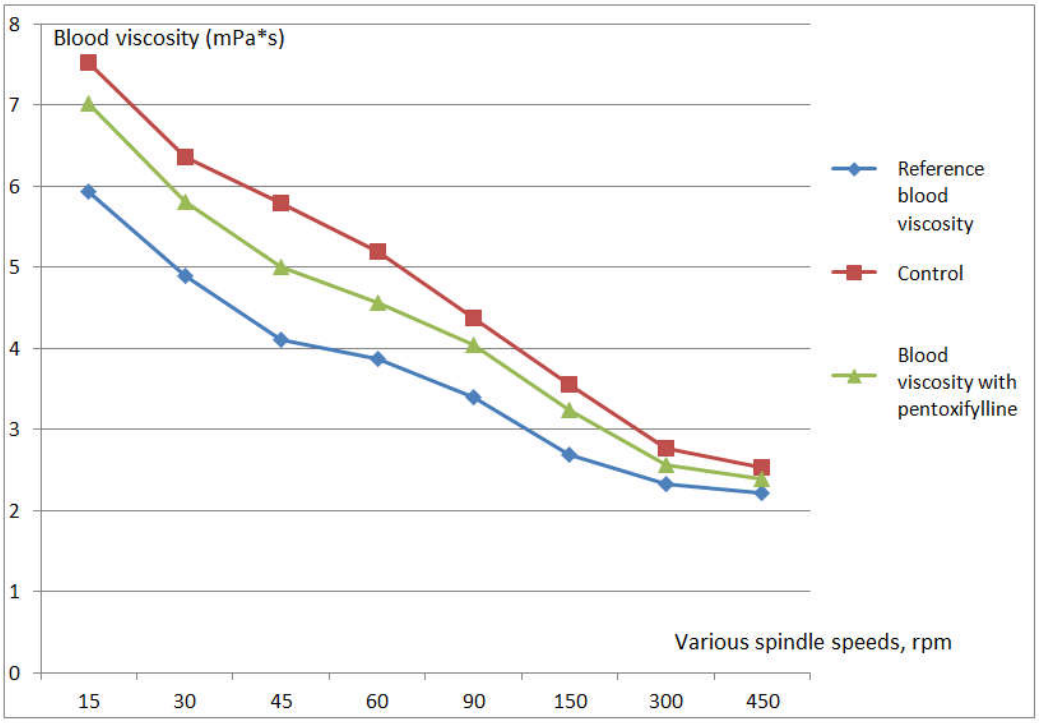

Figure 5. The hemorheological activity of pentoxifylline.

Thus, the obtained experimental data has demonstrated the following result of the in vitro biological screening for the hemorheological activity that compound 2 has been able to reduce blood viscosity in the in vitro hyperviscosity model; and it has been as good as the reference drug pentoxifylline in the hemorheological effects in the in vitro hyperviscosity model.

3. Conclusions

As a result of this study, a new compound 4-[(3,5-dimethyl-1,2-oxazol-4-yl)sulfonyl]cytisin 2 has been synthesized and has been comprehensively investigated. Its structure has contained an isoxazole heterocycle, a sulfogroup, and an alkaloid cytisine.

1. Structures of compounds of 2 and 3 have been determined with the 1H, 13C NMR spectroscopy.

2. 2D NMR spectroscopy of COZY (1H-1H), HMQC (1H-13C) and HMBC (1H-13C) has been used to study the mutual influence of atoms inside molecules of 2 and 3.

3. The X-ray diffraction analysis has established a spatial structure of compound 2. All parameters of a crystal structure and the structural features have been determined.

4. Reaction with p-chlorobenzaldehyde has defined that a methyl group at 5-position of the 4-sulfamidisoxazole cycle has been more active in condensation reaction with the substituted aromatic aldehydes.

5. The biological screening has showed a high hemorheological activity of 4-[(3,5-dimethyl-1,2-oxazol-4-yl)sulfonyl]cytisine 2. It has been as good as a well-known angioprotector pentoxifylline.

4. Experimental

^{1}H and ^{13}C NMR spectra have been taken on JNM-ECA Jeol 400 spectrometer (frequency 399.78 and 100.53 MHz, respectively) with using of $CDCl_3$ solvent. The chemical shifts have been measured relative to signals of residual protons or carbon atoms of deuterated chloroform. A melting point of a substance has been determined on SMP10 device. The reaction and purity of an obtained compound have been monitored with the thin-layer chromatography on Silufol UV-254 plates in system of isopropyl alcohol-benzene-25% ammonia solution, 10:5:2. The plates have been exposed to iodine vapor.

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The X-ray diffraction experiment. The cell parameters and intensities of 8569 pictures (3147 independent, R_{int} = 0.029) have been measured on Bruker KARRA APEX2 CCD (MoK_α) diffractometer, graphite monochromator, φ,θ-scanning, 2.721°< θ < 25.990°) at a temperature of 296 K.

Crystals **2** has been monoclinic, a space group $P2_1$, a = 6.6291(3) Å, b = 8.9064(5) Å, c = 13.9604(7) Å, β = 98.093(2)°, V= 816.03(7) A^3, Z= 2 ($C_{16}H_{19}N_3O_4S$), M = 349.40 g/mol, $d_{calc.}$= 1.422 g/cm^3, μ = 0.225 mm^{-1}. The array of measured intensities has been processed. Absorption corrections have been performed using the SAINT [24] and SADABS [25] programs included in APEX2 software package (multiscan, T_{min} = 0.871, T_{max} = 0.904).

Structure has been decoded with a direct method. Positions of the non-hydrogen atoms have been clarified in the anisotropic approximation by the full matrix MNA. Hydrogen atoms have been placed in the geometrically calculated positions. Their positions have been refined in the isotropic approximation with the fixed positional and thermal parameters (a "rider" model). Calculations have used 2944 independent pictures with I>2σ(I). The number of the refined parameters has been 219. Final divergence factors have been R1 = 0.0317, wR_2 = 0.0749 for pictures with I>2σ(I), R_1= 0.0359, wR_2 = 0.0784 for all pictures, GOOF = 0.990. The residual density peaks: Δρ = 0.184 e/A^3 and -0.214 e/A^3. Structure has been decoded and refined with using the programs of SHELXT-2014/5 [26] and SHELXL-2018/3 [27]. The X-ray diffraction data in the form of a CIF file has been deposited in the Cambridge Crystallographic Data Center (CCDC 2168324).

4.1. Experimental Procedures

3,5-Dimethylisoxazole-4-sulfonyl chloride (1). To a cooled mixture of 33.8 mL of a chlorosulfonic acid and 4.06 mL of thionyl chloride, 5 mL of 3,5-dimethylisoxazole has been slowly added under stirring. A reaction mixture under stirring has been slowly heated to 120-130°C for 4 h.

The reaction mixture has been cooled to a room temperature and poured over 100 g of ice (be careful when added to ice, a reaction mixture can react vigorously with water).

The precipitated white residue has been filtered, washed with water and dissolved in 30 ml of chloroform. Solution has been washed with 40 ml of potassium carbonate 5% solution and dried over calcium chloride. Product (9) has been obtained as the white crystals. Its yield has been 2.28 g, m.p. 40-2°C.

4-[(3,5-dimethyl-1,2-oxazol-4-yl)sulfonyl]cytisine (2). To a solution of 1.9 g (0.01 mol) of cytisine and 1.18 g (0.015 mol) of pyridine in 3 ml of dry acetonitrile, 1.95 g (0.01 mol) of sulfochloride 2 in 10 ml of dry acetonitrile has been added at a room temperature. A color of a reaction mixture has been light yellow, and its residue has been yellow. A reaction mixture has been stirred for 1 h at 40°C. Then a reaction mixture has been cooled. A yellow residue has been filtered and washed with acetonitrile. Then a solvent has been distilled with using a rotary evaporator. A residue has been thick yellow oil. Then 20 ml of a 5% solution of potassium carbonate has been added to a residue. After grinding, the thick oil has been a yellowish powder. The yield of product 2 has been 83%, m.p. 141-142°C.

4-[(3-methyl-5-{(4-chlorophenyl)ethenyl}-1,2-oxazol-4-yl)sulfonyl]cytisine (3). To a solution of 0.91 g of sulfonamide 2 and 0.56 g of 4-chlorobenzaldehyde in 20 ml of ethanol, 2 ml of a 40% aqueous solution of potassium hydroxide has been added at a room temperature. A color of a reaction mixture has been cloudy, and then has become light yellow. A reaction mixture has been stirred and heated for 1 h at 60-70°C. Then, a reaction mixture has been cooled to a room temperature and added 15% hydrochloric acid solution to pH≤3. A residue has been filtered and washed with water. The residue was a hygroscopic substance, light yellow color, m.p. 217-219°C.

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Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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