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The redox transformations and nucleophilic replacements as possible metabolic reactions of the drug "Triazaverin". The chemical modeling of the metabolic processes

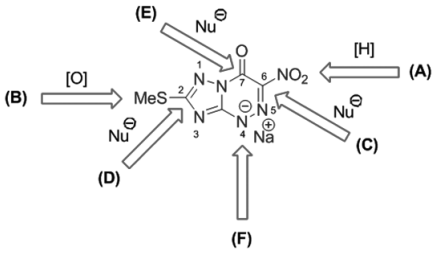
As a model of metabolic transformations of antiviral drug "Triazaverin" and its analogues-2-alkylthio-6-nitro-1,2,4-triazolo[5,1-c][1,2,4]triazine-7-ones 1a-d examined the oxidation of alkylthio groups to the corresponding sulfoxides 2a-d and sulfones 3a-d, as well as the process of nucleophilic substitution sulfonyloxy group of cysteine and cysteamine with the formation of compounds 5 and 6.

Key words: biological active compounds; heterocycles; triazine; Triazaverin; antiviral drug.

Introduction

The relevance of creating new antiviral drugs due to the joint action of such operating factors as the spread of socially significant, particularly dangerous infections, and the emergence of pathogenic viral strains resistant to existing drugs.

The antiviral drug "Triazaverin" and its analogues are highly effective in experiments *in vivo* and decreased activity in experiments on cell cultures, suggesting that the antiviral effect is not the "Triazaverin", and products of its transformations in the body. One way to identify



such transformations in the organism is to predict the possible products of modifications of the compounds and chemical synthesis models.

Based on the molecular structure of compounds 1a and study of chemi-

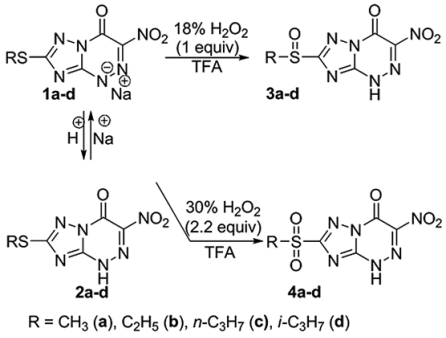
cal properties of nitroazolo[5,1-c][1,2,4] triazines [1] can assume various variants of metabolism: redox transformations in the body (directions A, B) as the reduction of the nitro group under the action of a reductases (direction A), oxidation of alkylthio fragment under the action of oxidases (direction B) and its further transformation. The part transferases under the action of N – and S-nucleophils, such as lysine, arginine, cysteine could lead to the replacement of alkylthio- or nitro groups (directions C, D).

The hydrolytic enzymes are also quite capable to lead the transformation of "Triazaverin" accompanying the degradation of triazine cycle with the break of bond C-N (direction E). The alkylation on the N-atom is possible too (direction F).

With regard to the redox transformations of "Triazaverin" that we previously established that after the intragastric administration of laboratory animals of the drug substance, there is a reduction of the nitro group with the formation of 2-methylthio-6-amino-1,2,4-triazolo[5,1-c][1,2,4]triazine-7-one [2], does not exhibit antiviral action *in vitro* and, most likely, which is not applicable metabolite.

Results and Discussion

Considering the possibility of oxidation of alkylthio groups under use "Triazaverin" and its analogues, it should be noted that in the majority of viral infections at an early stage of the process is increased generation of reactive oxygen species [3]. Based on the foregoing, it is natural to assume that in these conditions can occur in the oxidation reaction of S-methyl group of "Triazaverin" characteristic, for example, for SH-containing amino acids of methionine under oxidative stress.



Scheme 1

In the present communication present data on the production model experiments with the drug "Triazaverin" – the study of the oxidation of S-methyl group and the behavior of oxidation products under the action of S-nucleophils. This approach allows, on the one hand, to predict the course of chemical reactions related to the behavior of the drug in the body, and on the other hand, by the synthesis of the respective compounds to simulate the process of formation of covalent bonds of azolo[5,1-c]1,2,4-triazines with S-key fragments of proteins as cells and virus.

Redox-transformations of "Triazaverin" and its derivatives

The first aspect of this work was the synthesis of model compounds – oxidation of sulfhydryl groups in molecules of sodium salts of 2-alkylthio-6-nitro-1,2,4-triazolo[5,1-c]1,2,4-triazine-7-ones **1a-d** or in the associated N-H-acids comprising the formation of heterocyclic sulfoxides **3a-d** and sulfones **4a-d**. Sulfoxides

3a-d (Scheme 1) were obtained under the treatment of the compounds **1a-d** or 2a-d by the equimolar quantity of 18 % hydrogen peroxide in the trifluoroacetic acid.

Further oxidation to the corresponding sulfones **4a-d** with the yields 62–71 % carried out by gradual addition of excess 2.2 equivalent of 30 % hydrogen peroxide to the suspension of 2-alkylthio-1,2,4-triazolo[5,1-c]-triazines in the trifluoroacetic acid at room temperature.

The represented reactions model the possible metabolic transformations of "Triazaverin" and its derivatives under the action of the active forms of oxygen including hydrogen peroxide.

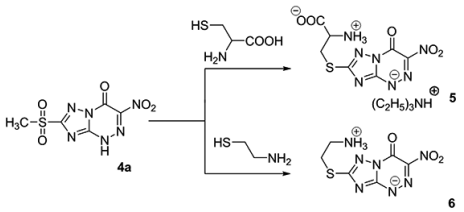
Nucleophilic replacement of methylsulfonilic group. It is known that alkylsulfonyl fragments are susceptible to the substitution reactions when interacting with nucleophiles [4]. With regard to the behavior of nitro group in "Triazaverin" but the susceptibility of the nitro group associated with aromatic (heteroaromatic) cycle to the displacement under the nucleophiles [5] is well known.

Thus in the structure of 2-alkylsulfonyl-1,2,4-triazolo[5,1-c]1,2,4-triazine-7-ones **4a-d** there are two groups that are susceptible to nucleophilic displacement. This situation is of interest for the research of the comparative reactive ability of the easy outgoing groups CH_3SO_2- in 1,2,4-triazole and NO_2 in1,2,4-triazine cycles in the compounds 4a-d and also as the model of the behavior of "Triazaverin" in the organism. As the nucleophilic reagents in this work were used S-nucleophiles(cysteine and cysteamine), which can be regarded as models of protein fragments containing cysteine fragment.

The substitution sulfonylic fragment in 2-methylsulfonyl-1,2,4-triazolo[5,1-c]1,2,4-triazine **4a** under the influence, as cysteine and cysteamine, occurs in dry methanol in the presence of triethylamine in a few days with the formation of compounds 5 and 6- substitution products methylsulfonyl group with the yields 41–46 % (Scheme 2).

The date of NMR ^{1}H, IR-spectroscopy and elemental analysis for the compounds 5, 6 correspond to the attributed structures.

The described displacement of alkylsulfonyl group in the compound **4a** is the argument in favor of the assumption about the possible participation of this fragment in the metabolic transformations of "Triazaverin".



Scheme 2

Experiment part

NMR^{1}H and ^{13}C spectra were recorded on a spectrometer Bruker DRX-400 (400 and 100 MHz, respectively) in DMSO-d_6 and D_2O. The chemical shifts are recorded in σ scale relatively internal standard TMS for NMR^{1}H spectra. Elemental analyses were performed on CHNS-analyzer "Perkin Elmer 2400-II". IR spectra (4000–400 cm^{-1}) of the received compounds were recorded on a spectrophotometer "Perkin Elmer Spectrum One BFTIR" in thin layer of a

sample (DRA). Reaction monitoring and the individuality of the synthesized compounds was performed by TLC plates Sorbfil in the systems: ethyl acetate and butanol-acetic acid-water 4:1:1.

General methods 1 for preparation of 2-alkylsulfinyl-6-nitro-1,2,4-triazolo[5,1-c]1,2,4-triazin-7-ones (3a-d).

To a suspension 0.01 mol of sodium salt 2-alkylthio-6-nitro-1,2,4-triazolo[5,1-c]1,2,4-triazin-7-one (1) in 10 mL of trifluoroacetic acid by mixing was added 1.78 ml (1 eq.) of 18 % hydrogen peroxide. The reaction mass is mixed at room temperature during 3 hours. The resulting solid precipitate was filtered and was crystallized from iso-propanol.

2-Methylsulfinyl-6-nitro-1,2,4-triazolo[5,1-c]1,2,4-triazine-7-one (3a) was obtained on the general methods 1 from compound 1a in terms of beige crystalline solid, yield 70 %, mp 256 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): 9.84 (1H, уш.с., NH), 3.05 (3H, с, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 169.09 (C_2), 157.32 (C_{3a}), 144.05 (C_7), 143.57 (C_6), 25.58 (CH_3SO); IR (ν/sm^{-1}): 1750 (C=O); 1036 (–SO-); 1553,1340 (NO_2); elemental analysis, calculated for $C_5H_4N_6O_4S$ %: C 24.59, H 1.65, N 34.42. Found, %: C 24.62, H 1.43, N 34.28.

2-Ethylsulfinyl-6-nitro-1,2,4-triazolo[5,1-c]1,2,4-triazine-7-one (3b) was obtained on the general methods 1 from compound 1b in terms of beige crystalline solid. Yield 74 %, mp 227 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): 11.16 (1H, br.s., NH), 3.36–3.16 (2H, m, CH_2), 1.24 (3H, t, J = 7.4, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 168.34 (C_2), 157.58 (C_{3a}), 144.36 (C_7), 143.87 (C_6), 46.64 (CH_2SO), 6.15 (CH_3); IR (ν/sm^{-1}): 1748 (C=O); 1022 (–SO-); 1552, 1336 (NO_2); elemental analysis, calculated for $C_6H_6N_6O_4S$, %: C 27.91, H 2.34, N 32.55. Found, %: C 27.87, H 2.27, N 32.31.

2-Propylsulfinyl-6-nitro-1,2,4-triazolo[5,1-c]1,2,4-triazine-7-one (3c) was obtained on the general methods 1 from compound 1c in terms of beige crystalline solid. Yield 72 %, mp 222 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): 8.41 (1H, br.s., NH), 3.29–3.18 (2H, m, $SOCH_2$), 1.78–1.55 (2H, m, CH_2), 1.01 (3H, t, J = 7.4, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 168.19 (C_2), 157.87 (C_{3a}), 144.19 (C_7), 143.46 (C_6), 54.15 (CH_2SO), 15.21 (CH_2), 12.94 (CH_3); IR (ν/sm^{-1}): 1748 (C=O); 1013 (–SO-); 1556, 1336 (NO_2); elemental analysis, calculated for $C_7H_8N_6O_4S$, %: C 30.88, H 2.96, N 30.87. Found, %: C 30.82, H 3.12, N 30.77.

2-iso-Propylsulfinyl-6-nitro-1,2,4-triazolo[5,1-c]1,2,4-triazine-7-one (3d) was obtained on the general methods 1 from compound 1d in terms of beige crystalline solid. Yield 77 %, mp 239 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): 7.60 (1H, br.s., NH), 3.49–3.38 (1H, m, CH), 1.27 (6H, dd, J = 6.8, $2CH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz): 167.79 (C_2), 157.42 (C_{3a}), 144.33 (C_7), 143.86 (C_6), 53.07 (CHSO), 15.92 (CH_3), 14.59 (CH_3); IR (ν/sm^{-1}): 1752 (C=O); 992(–SO$^-$); 1555, 1342 (NO_2); elemental analysis, calculated for $C_7H_8N_6O_4S$, %: C 30.88, H 2.96, N 30.87. Found, %: C 31.07, H 2.95, N 30.89.

The general methods 2 for preparation of 2-alkylsulfonyl-6-nitro-1,2,4-triazolo[5,1-c]1,2,4-triazin-7-ones (4a-d).

To a suspension 0.01 mol of sodium salt 2-alkylthio-6-nitro-1,2,4-triazolo[5,1-c]1,2,4-triazin-7-one (1) in 14 mL of trifluoroacetic acid by mixing dropwise was added 4 ml (2 eq.) of 30 % hydrogen peroxide in order that a temperature wasn't

above 80 °C. Then the reaction mass was mixed at a room temperature during 3 hours, the precipitate was filtered and crystallized from *iso*-propanol.

2-Methylculfonyl-6-nitro1,2,4-triazolo[5,1-c]1,2,4-triazine-7-one (4a) was obtained on the general methods 2 from compound 1a in terms of beige crystalline solid. Yield 66 %, mp 275 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): 7.57 (1H, br.s., NH), 3.41 (3H, s, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 163.86 (C_2), 158.41 (C_{3a}), 144.69 (C_7), 143.64 (C_6), 41.84 (CH_3SO_2); IR (ν/sm^{-1}): 1759 (C=O); 1347, 1138 (–SO_2-); 1570, 1323 (NO_2);); elemental analysis, calculated for $C_5H_4N_6O_5S$, %: C 23.08, H 1.55, N 32.30. Found, %: C 23.21, H 1.31, N 32.33.

2-Ethylsulfonyl-6-nitro-1,2,4-triazolo[5,1-c]1,2,4-triazine-7-one (4b) was obtained on the general methods 2 from compound 1b in terms of beige crystalline solid. Yield 64 %, mp 259 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): 9.71 (1H, br.s., NH), 3.52 (2H, qv., J = 7.4, CH_2), 1.33 (3H, t, J = 7.4, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 162.82 (C_2), 158.93 (C_{3a}), 145.06 (C_7), 144.02 (C_6), 48.54 (CH_2SO_2), 7.25 (CH_3); IR (ν/sm^{-1}): 1759 (C=O); 1311, 1140 (–SO_2-); 1557, 1332 (NO_2); elemental analysis, calculated for $C_6H_6N_6O_5S$, %: C 26.28, H 2.21, N 30.65. Found, %: C 26.44, H 2.20, N 30.43.

2-Propylsulfonyl-6-nitro-1,2,4-triazolo[5,1-c]1,2,4-triazine-7-one (4c) was obtained on the general methods 2 from compound 1c in terms of beige crystalline solid. Yield 71 %, mp 264 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): 11.98 (1H, br.s., NH), 3.53 (2H, t, J = 7.6, SO_2CH_2), 1.76–1.67 (2H, m, CH_2), 0.98 (3H, t, J = 7.4, CH_3); ^{13}C NMR (DMSO-d_6, 100 MHz): 163.22 (C_2), 158.44 (C_{3a}), 144.91 (C_7), 143.99 (C_6), 55.35 (CH_2SO_2), 16.20 (CH_2), 12.94 (CH_3); IR (ν/sm^{-1}): 1748 (C=O); 1293, 1139 (–SO_2^-); 1556, 1326 (NO_2); elemental analysis, calculated for $C_7H_8N_6O_5S$, %: C 29.17, H 2.80, N 29.16. Found, %: C 29.01, H 2.88, N 29.24.

2-iso-Propylsulfinyl-6-nitro-1,2,4-triazolo[5,1-c]1,2,4-triazine-7-one (4d) was obtained on the general methods 2 from compound 1d in terms of beige crystalline solid. Yield 62 %, mp 282 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): 8.27 (1H, br.s., NH), 3.73–3.63 (1H, m, CH), 1.35 (6H, d, J = 6.8, $2CH_3$); ^{13}C NMR (DMSO-d_6, 100 MHz): 161.91 (C_2), 158.80 (C_{3a}), 144.98 (C_7), 144.02 (C_6), 54.30 ($CHSO_2$), 15.02 ($2CH_3$); IR (ν/sm^{-1}): 1749 (C=O); 1311, 1135 (–SO_2-); 1556, 1326 (NO_2); elemental analysis, calculated for $C_7H_8N_6O_5S$, %: C 29.17, H 2.80, N 29.16. Found, %: C 29.11, H 2.69, N 29.00.

The general methods 3 of nucleophilic replacement in 2-methylsulfonyl-6-nitro-1,2,4-triazolo[5,1-c]1,2,4-triazin-7-one.

To a suspension 0.001 mol of cysteine (or cysteamine) in 20 ml of methanol were added triethylamine and were mixed at the argon atmosphere during 5 min, then to a reaction mass were added equivalent of 2-alkylsulfonyl-triazolotriazin and were refluxed. The end of reaction is determined by TLC in system: butanol-acetic acid-water 4:1:1. The resulting solid precipitate was washed with *iso*-propanol.

Triethylammonium salt of (2'-amino-2'-carboethoxyethylthio)-6-nitro-1,2,4-triazolo[5,1-c]triazine hydrochloride (5) was obtained with use of the general methods 3 and 3 equivalent of triethylamine. The product was crystallized from the water ethanol in terms of canary crystalline solid. Yield 46 %,

mp 158 °C, ^{1}H NMR (D_2O, 400 MHz): 4.27 (1H, dd, *J* = 7.5, 3.8, CHN), 3.98 (1H, dd, *J* = 15.2, 3.8, H_a in SCH_2), 3.66 (1H, dd, *J* = 15.2, 7.5, H_b in SCH_2), 3.21 (6H, qv., *J* = 7.28, $3CH_2$), 1.29 (9H, t, *J* = 7.28, $3CH_3$); ^{13}C NMR (D_2O, 100 MHz): 172.02 (COO^-), 166.09 (C_2), 159.16 (C_{3a}), 144.55 (C_7), 143.06 (C_6), 54.47 (CHN), 46.71 ($3CH_2$), 31.67 (SCH_2), 8.27 ($3CH_3$); IR (ν/sm^{-1}): 1682, 1615 (C=O); 1504, 1361 (NO_2); elemental analysis, calculated for $C_{13}H_{22}N_8O_5S*H_2O$, %: C 37.10, H 5.71, N 26.63. Found, %: C 36.96, H 5.56, N 26.72.

2-(2'-Amino-ethylthio)-6-nitro-1,2,4-triazolo[5,1-c]1,2,4-triazine hydrate (6) was obtained with use of the general methods 3 and 2 equivalent of triethylamine. The product was crystallized from the water ethanol in terms of canary crystalline solid. Yield 41 %, mp 285 °C, ^{1}H NMR (D_2O, 400 MHz): 3.58 (2H, t, $J = 7.03, NCH_2$), 3.49 (2H, t, $J = 7.03, SCH_2$); ^{13}C NMR (DMSO-d_6, 100 MHz): 163.95 (C_2), 160.12 (C_{3a}), 144.77 (C_7), 142.94 (C_6), 38.69 (CH_2N), 27.87 (SCH_2); IR (ν/sm^{-1}): 1690 (C=O); 1514, 1371 (NO_2); 3521 (broadened) ($-NH_3^+$); elemental analysis, calculated for $C_6H_7N_7O_3S*H_2O$, %: C 26.18, H 3.27, N 35.64. Found, %: C 25.91, H 2.98, N 35

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