Synthesis of Bis(Isoxazol-4-Ylmethylsulfanyl)Alkanes and Some Metal Complexes as a Hepatoprotective Agents

Vnira Rakhimovna Akhmetova, Rozalia Akramovna Galimova, Nail Salavatovich Akhmadiev, Albina Midkhatovna Galimova, Ravil Akhmetzyanovich Khisamutdinov, Galiya Maratovna Nurtdinova, Eduard Feliksovich Agletdinov, Valery Alekseevich Kataev

1 Institute of Petrochemistry and Catalysis, Russian Academy of Sciences, 141 Prospekt Oktyabrya, 450075 Ufa, Russia.
2 Bashkir State Medical University, 3 Lenin Str., 450008 Ufa, Russia.
3 Ufa Institute of Chemistry, Russian Academy of Sciences, 71 Prospekt Oktyabrya, 450054 Ufa, Russia.

Abstract

Purpose: This research is devoted to designing the synthesis of sulfanyl-substituted 3,5-dimethylisoxazoles, which contain structural analogues of the SAM drug in the molecule. SAM (S-adenosyl-L-methionine), formed in the biosynthetic process, is used as an effective hepatoprotective drug. Complexation and hepatoprotective properties of the combinatorial series of bis(isoxazolylsulfanyl)ethane have been studied.

Methods: Bis(isoxazol-4-ylmethylsulfanyl)alkanes were synthesized using the one-pot method. The structures of compounds were established by one-dimensional (1H, 13C) and two-dimensional (COSY, HMQC, HMBC) NMR spectroscopy, mass-spectrometry and X-ray diffraction. The biological activity of the combinatorial series of sulfanyl derivatives of diketones, azoles and their metal complexes has been studied by in vivo method. Simulation of the animal associated processes was carried out in accordance with the principles of bioethics. Screening studies of hepatoprotective activity were carried out in a model of acute CCl4 intoxication after a single injection intraperitoneally as a 50% solution in olive oil. The pharmacologically known hepatoprotective drug SAM served as a control.

Results: Two-step synthesis of novel a,ω-bis(isoxazol-4-ylmethylsulfanyl)alkanes was carried out via the multicomponent reaction between 2,4-pentandione, CH3O and a,o-dithiols, then the resulting a,ω-bis(1,3-diketone-2-ylmethylsulfanyl)alkanes were transformed by hydroxyl amine to obtain bis-isoxazole derivatives. Promising precursor 1,2-bis(isoxazol-4-ylmethylsulfanyl)ethane was converted to metal complexes by interaction with PdCl2 or CuCl. The obtained compounds were found to be practically non-toxic compounds (1001 – 3000 mg/kg) according to the classification of K.K. Sidorov, but copper complex refers to low-toxic compounds substances (165 mg/kg). Compounds of sulfanyl ethane series demonstrate hepatoprotective activity.

Conclusion: Palladium(II) complex being almost non-toxic possesses hepatoprotective activity comparable to the drug like SAM.

Introduction

Currently the use of organometallic complexes in medicine is considered as an innovative approach, due to their unusual activity in biological systems.1-3 From the standpoint of metal-ligand homeostasis, organic complexes with essential metals forming part of the active site of many enzymes,4,7 are very promising for the treatment of pathological states.8 Recently9 it has been found that baicalin-copper complex is effective hepatoprotective agent unlike baicalin itself.

Breakthrough event was the discovery in the late 20th-century therapeutic properties of cisplatin,10 and other platinum complexes against cancer.11,12 which, unfortunately, have significant toxic side effects.13,14 Later it was shown that the less toxic palladium(II) complexes were also effective for the treatment of cancer. Nowadays there is the interest of researchers to look for low-toxic organometallic complexes with an effective anti-tumor15-18 or hepatoprotective activity.19 We have previously reported the one-pot effective synthesis of a,ω-bis(1,3-diketone-2-ylmethylsulfanyl)alkanes,20 which are promising precursors for methylsulfanyl substituted a,ω-bis-pyrazoles with pronounced inhibitory effects on alphamylase activity.21-23 Taking into account, that isoxazoles exhibit pharmacological properties,24-27 and sulfanyl substituted isoxazoles are polydentate ligands,28-29 our aim was to carry out the synthesis of novel complexes of Pd(II) and Cu(I) with 1,2-bis[3,5-dimethylisoxazol-4-yl]methylsulfanyl] ligands and examine the toxicological
and hepatoprotective properties of metallo-complexes and precursors thereof.

Materials and Methods

General procedures and materials

The reaction products were characterized by 1H and 13C NMR spectra that were recorded on spectrometers Bruker Avance 400 NMR (400.13 MHz and 100.62 MHz) and Bruker Ascend III HD 500 (500.17 MHz and 125.78 MHz), internal standard TMS, solvent DMSO-d6. The homo- and heteronuclear 2D experiments were performed by the standard pulse sequences of Bruker.

IR spectra were recorded on a Bruker Vertex-70 FTIR and Specord M80 spectrometers. Electrospray ionization (ESI) mass spectra were obtained on a HPLC mass spectrometer LCMS-2010EV (Shimadzu) in positive and negative ions mode at the corona discharge needle ionizing electrode and ionizing capillary potential of −3.5 kV. Sample solution (direct syringe sample inlet) under ESI conditions was in methanol (acetonitrile), mobile phase was acetonitrile/water, 95/5. Mass-spectra was recorded on a device MALDI TOF Autoflex III firm Bruker (compounds 2a-e) with sinapinic acid as a matrix (see Supplementary data).

Elemental analysis was performed on a Carlo Erba 1106 elemental analyzer. Melting points were determined on a Kofler hot-stage microscope and utilized uncorrected. Individuality and purity of synthesized compounds were controlled by means of TLC on Silufol UV-254 plates; I2 was used as a developer.

General procedure of thiomythylation of 2,4-pentanedione with formaldehyde and α,ω-dithiols. 1,2-Bis[(pentane-2,4-dione-3-yl)methylsulfanyl]alkanes (2a–e)

In Schlenk vessel using a magnetic stir bar was added with formaldehyde (37% aqueous solution, 20 mmol, 1.47 mL) and α,ω-dithiol (10 mmol) stirred for 30 min in argon atmosphere. Then 2,4-pentanedione (20 mmol) and the promoter BuONa (10 mmol) in 5 mL CHCl3-C2H5OH (1:1) were added. The mixture was stirred for 1 h at r.t. The precipitate was filtered, washed with alcohol to give the target product 1a: (81%, 2.58 g) as a white crystals, mp 139–141°C (data lit. 138–140°C). The spectra of other sulfanyl derivatives of bis-diketones are similar to those previously obtained.

Synthesis of 1,2-bis[(3,5-dimethylisoxazol-4-yl)methylsulfanyl]alkanes (2a–e) (General method)

Sulfanyl-substituted bis-diketones (10 mmol), 15 mL of ethanol were added into the glass vessel, was added with small portions of hydroxylamine (25 mmol, 1.74 g). The reaction mixture was heated up to 60°C and stirred for 2 h. Then formed precipitate was filtered, washed with water (2 x 15 mL), and dried in open air. 1,2-Bis[(3,5-dimethylisoxazol-4-yl)methylsulfanyl]ethane (2a) Yield: 97%; white crystals, mp: 155–156°C (data lit. 154–156°C).

1,3-Bis[(3,5-dimethylisoxazol-4-yl)methylsulfanyl]propane (2b) white solide (56%); Rf=0.59 (1:2:10 cyclohexane/CH2Cl2/EtOAc); mp 84–86°C; IR (thin film) νmax 1632, 1190, 1034, 886, 738 cm−1; 1H NMR (DMSO-d6, 500 MHz) δ=3.53 (4H, s, CH2); 2.48 (4H, t, 3J = 7.2 Hz, H-9, -11); 2.33 (6H, s, CH3); 2.20 (6H, s, CH3); 1.77 (2H, p, J = 7.2 Hz, CH2); 13C NMR (DMSO-d6, 125 MHz) δ=166.1 (C, C-3, -15), 159.6 (C, C-5, -18), 111.3 (C, C-4, -14), 30.2 (CH2, C-9, -11), 28.9 (CH2, C-10), 22.8 (CH2, C-7, -13), 10.9 (CH2, C-21, -19), 10.1 (CH2, C-6, -20); MALDI TOF m/z 327.327 C10H12N2O4S (cald. 327.485); 349.267 C10H17N2O4S2Na (cald. 349.476); Anal. Calcd. for C10H12N2O4S2: C, 55.18; H, 6.79; N, 8.58; S, 19.64.

Found: C, 55.34; H, 6.85; N, 8.42; S, 19.71.

1,4-Bis[(3,5-dimethylisoxazol-4-yl)methylsulfanyl]butane (2c) white solide (94%); Rf=0.61 (1:2:10 cyclohexane/CH2Cl2/EtOAc); mp 68–70°C; IR (thin film) νmax 1637, 1192, 1032, 893, 719 cm−1; 1H NMR (DMSO-d6, 500 MHz) δ=3.45 (4H, s, CH2); 2.40 (4H, m, CH2); 2.32 (6H, s, CH3); 2.19 (6H, s, CH3); 1.57 (4H, m, CH2); 13C NMR (DMSO-d6, 125 MHz) δ=166.0 (C, C-3, -15), 159.7 (C, C-5, -19), 111.4 (C, C-4, -15), 30.7 (CH2, C-9, -12), 28.4 (CH2, C-10, -11), 22.7 (CH2, C-7, -14), 10.9 (CH3, C-20, -22), 10.1 (CH3, C-6, -21); MALDI TOF m/z 363.341 C10H14N2O4S2Na (cald. 363.494); Anal. Calcd. for C10H14N2O4S2: C, 56.44; H, 7.10; N, 8.23; S, 18.83. Found: C, 56.49; H, 7.21; N, 19.89; S, 18.97.

1,5-Bis[(3,5-dimethylisoxazol-4-yl)methylsulfanyl]-3-thiapentane (2d) white solide (74%); Rf=0.66 (1:2:10 cyclohexane/CH2Cl2/EtOAc); mp 71–73°C; IR (thin film) νmax 3421 (N–H), 1633 (C=O), 1192 (C–N), 726 (C–S) cm−1; 1H NMR (DMSO-d6, 500 MHz) δ=3.61 (4H, s, CH2); 2.74 – 2.61 (8H, m, SC6H5SC6H5); 2.34 (6H, s, CH3); 2.20 (6H, s, CH3); 13C NMR (DMSO-d6, 125 MHz) δ=166.2 (C, C-3, -15), 159.7 (C, C-5, -19), 111.4 (C, C-4, -15), 30.7 (CH2, C-9, -12), 28.4 (CH2, C-10, -11), 22.7 (CH2, C-7, -14), 10.9 (CH3, C-20, -22), 10.1 (CH3, C-6, -21); MALDI TOF m/z 395.041 C10H12N2O4S2Na (cald. 395.588); 411.004 C10H14N2O4S2K (cald. 411.667); Anal. Calcd. for C10H14N2O4S2: C, 51.58; H, 6.49; N, 7.52; S, 25.82. Found: C, 51.67; H, 6.53; N, 7.64; S, 26.13.

1,6-Bis[(3,5-dimethylisoxazol-4-yl)methylsulfanyl]hexane (2e) white solide (69%); Rf=0.59 (1:2:10 cyclohexanes/CH2Cl2/EtOAc); mp 76–78°C; IR (thin film) νmax 3436, 1634, 1196, 1038, 721 cm−1; 1H NMR (DMSO-d6, 500 MHz) δ=3.52 (4H, s, CH2); 2.39 (4H, t, 3J = 7.2 Hz, CH2S); 2.33 (6H, s, CH3); 2.20 (6H, s, CH3); 1.49 (4H, m, CH2); 1.30 (4H, m, CH2); 13C NMR (DMSO-d6, 125 MHz) δ=165.9 (C, C-3, -18), 159.6 (C, C-5, -21), 111.4 (C, C-4, -17), 31.1 (CH2, C-9, -14), 29.2 (CH2, C-10, -13), 28.3 (CH2, C-11, -12), 22.8 (CH2, C-7, -16), 10.9 (CH3, C-22, -24), 10.1 (CH3, C-6, -23); MALDI TOF m/z calculated for 369.316 C10H14N2O4S2 (cald. 369.565); C10H14N2O4S2Na 391.267 (cald. 391.547); 407.222 C10H16N2O4S4K (cald. 407.655);
Anal. Calcd. for C_{18}H_{22}N_{3}S_{2}: C, 58.66; H, 7.66; N, 7.60; S, 17.40. Found: C, 58.87; H, 7.81; N, 7.54; S, 17.62.

Cis-S,S-dichloride-1,6-(3,5-dimethylisoxazol-4-yl)-2,5-dithiahexane palladium(II) complex (3)
In the glass vessel (1.405 mmol, 0.25 g) palladium(II) chloride was dissolved in 15 mL of acetonitrile by stirring at 60 °C. After cooling up to r.t. 1,2-bis(3,5-dimethylisoxazol-4-yl)methylsulfanyl]ethane (1.405 mmol, 0.44 g) was added and reaction mixture was stirred for 3 h. The resulting bright yellow precipitate was filtered through filter paper (blue ribbon) and washed by acetonitrile, water and dried in open air with formation yellow powder (345%); mp > 250°C (dec.); IR (thin film) ν_{max} 1635 (br), 1274, 1250, 1193, 883, 829, 715, 661, 334, 307 cm⁻¹; ¹H NMR (DMSO-d₆, 500 MHz) of diastereomeric mixture (AB system): δ=6.63 (2H, dd, J=14.4 Hz, CH₃), 4.33 (2H, dd, J=14.4 Hz, ICH₂), 4.47 (2H, dd, J=14.0 Hz, CH₃), 4.18 (2H, dd, J=14.0 Hz, CH₂), 3.51 (2H, dd, J=9.2 Hz, SCH₂S), 3.15 and 3.09 (2H, br s, SCH₂S), 2.93 (2H, dd, J=9.2 Hz, SCH₂S), 2.43 (6H, s, CH₆), 2.26 (6H, s, CH₆); ¹³C NMR (DMSO-d₆, 125 MHz) δ=168.9 (C, C-1), -16, 159.6 (C, C-5), -19, 107.8 (C-4), -15, 37.5 and 37.3 (CH₂, C-9), -10, 30.4 and 29.7 (CH₂, C-7, -14), 11.5 (CH₂, C-13,23), 10.3 (CH₃, C-6,22); ESI m/z 527 [M+Cl]⁻ (100); Anal. Calcd. for C_{18}H_{25}Cl₂N₂O₂P₂S₄: C, 34.33; H, 4.12; Cl, 14.48; N, 5.72; Pd, 21.73, S, 13.09. Found: C, 34.07; H, 3.84; Cl, 14.74; N, 5.82; Pd, 22.12, S, 13.10.

Crystal Structure Determination and Refinement
The X-ray diffraction experiments of 2c and 2d single-crystals were carried out by a Bruker SMART 1000 CCD area detector using graphite monochromated MoKα radiation at 100 K. All calculations were performed on an IBM PC/AT using the SHELXTL software Atomic coordinates, bond lengths, bond angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC). X-ray diffraction data of 2c and 2d single-crystal was collected on an XCalibur Eos diffractometer with graphite monochromated Mo-Kα radiation (λ=0.71073 Å). Collection and processing of data performed with using the program CrysalisPRO Oxford Diffraction Ltd., Version 1.171.36.20. The structure was solved by direct methods as implemented in the program SHELXS-97,30,31. The refinement was carried out using SHELXL-97. The structure was refined by a fullmatrix least-square technique using anisotropic thermal parameters for nonhydrogen atoms and a riding model for hydrogen atoms.

Crystallographic data for the structure of 2c can be deposited in the Cambridge Crystallographic Data Centre as a CIF deposition with file number CCDC 1545010. Copies of these data can be obtained free of charge on application to CCDC, 12, Union Road, Cambridge, CB2 1EZ, UK (fax: 44 1223 336033, e-mail: deposit@ccdc.cam.ac.uk) or from http://www.ccdc.cam.ac.uk/data_request/cif.

Crystallographic data for the structure of 2d can be deposited in the Cambridge Crystallographic Data Centre as a CIF deposition with file number CCDC 1545008. The data can be obtained free of change on application to CCDC, 12, Union Road, Cambridge, CB2 1EZ, UK (fax: 44 1223 336033, e-mail: deposit@ccdc.cam.ac.uk) or from http://www.ccdc.cam.ac.uk/data_request/cif.

Biological assay
Simulation of animal-related processes was carried out in accordance with rules of laboratory practice (GLP) and the ethical norms of the Geneva Convention (1971). Conditions of experiment and keeping animals were carried out according to modern requirements. The tissue was fixed in a 10% solution of neutral formalin for light-optical examination of the liver. For further histological examination it was used samples of 5-7 mm thickness which were cut from a large proportion of the liver by cross-sectional dissection and subjected to standard treatment on the histological complex MICROM (Carl Zeiss, Germany). Samples were dehydrated in alcohols with increasing concentration, followed by pouring into paraffin blocks.

The studies were carried out on mice with a line BALB/CJ weighing 20 – 23 g (mice are provided by the Bashkir State Medical University Vivarium, Ufa, Russia). The animals were kept in 10 cells in a cage in standard vivarium conditions at an air temperature of 18 – 22 °C and a relative humidity of 50 to 65%. During the process there were free access to water and feed (~ 5 g/day).

Results and Discussion
The key substrates for the three step synthesis of target metallo-complexes of Pd(II) and Cu(I) was α,ω-
bis[(pentane-2,4-dione-3-yl)methylsulfanyl]alkanes 1a-e produced by a n-BuONa mediated multicomponent reaction (MCR) between 2,4-pentanedion, CH₂O and α,ω-dithiols. The yield of products 1a-e was decreased with increasing the aliphatic chain of α,ω-dithiols from 97 to 54%. Substrates 1a-e was successfully converted into α,ω-bis[(sulfanyl)methyl(3,5-methylisoxazol-4-yl)]alkanes 2a-e with high yields through the interaction between 1a-e and hydroxylamine hydrochloride in refluxing ethanol during 2 h (Figure 1). It should be noted, that in the indicated above two-step sequence, the total yield of 2a is higher than in the case of one-pot four-component reaction between 2,4-pentandione, CH₂O, 1,2-ethanedithiol and NH₂OH·HCl.

According to X-ray data (Figure 2), compound 2c is crystallized in the monoclinic and 2d - in orthorhombic crystalline system. It was found that bis(3,5-dimethylisoxazol) rings are in the cis-conformation with respect to S-(C)n-S fragment for compound 2c and trans-configuration for compound 2d. The crystallographic data for compounds 2c and 2d are collected in Table 1.

Among the synthesized 1,2-bis[(sulfanyl)methyl(3,5-dimethylisoxazol-4-yl)]ethanes 2a-e, 2a is the most promising for practical application taking into account the production efficiency and availability of the starting reagents. So that, 1,2-bis[(sulfanyl)methyl(3,5-dimethylisoxazol-4-yl)]ethane 2a was then transformed to Pd(II) complex 3 by reaction with PdCl₂ in CH₃CN. According NMR analysis, Pd(II) complex 3 in solution is the mixture of diastereomers (see Supplementary data). Using CuCl under the same reaction conditions Cu(I) complex 4 was also produced in 97% yield (Figure 3). According elemental analysis for complex 3 the ligand-metal ratio was 1:1 and for 4 as 1:2. In IR spectra of complexes 3 and 4 there are signals of M-S bonds is Pd-S in region 334 cm⁻¹ and Cu-S in region 320 cm⁻¹.

Biology
Combinatorial series of compounds 1a, 2a, 3, and 4 having the same alkylsulfanyl chain and different cyclic fragments of substitutes were assessed as hepatoprotective agents. They were used as solutions in TWIN oil. As known, the SAM drug (S-adenosyl-L-methionine 5, Figure 4) being produced via biosynthetic process is used for treatment of large group of diseases associated with the hepatotoxic action of the chemicals or the alcohol causing morphological changes in liver tissue, metabolism disorder or the toxic liver damages. SAM drug is considered as non-toxic sulfanyl-containing hepatoprotectors. It provides a stability of hepatocytes. Moreover, the transsulfuration (synthesis and turnover of glutathione and taurine, as well as conjugation and detoxication of bile acids and other xenobiotics), aminoproliliration and transmethylation processes are activated. Obviously SAM is a powerful antioxidant due to its sulfur atoms and heterocyclic fragments in the structure. As seen, compounds 2a-e, 3, 4 also contain these units.
Thus, we have used SAM as the object of comparison to study toxicity and hepatoprotective activity of the compounds 1a, 2a, 3 and 4.

| Compounds | 2c | 2d |
|-----------|----|----|
| Empirical formula | C_{16}H_{24}N_{2}O_{2}S_{2} | C_{16}H_{24}N_{2}O_{2}S_{3} |
| Formula weight | 340.49 | 372.55 |
| T/K | 298 | 298 |
| Crystal system | orthorhombic | monoclinic |
| Space group | Pbcn | P2_1/c |
| a/Å | 17.514(2) | 5.0140(7) |
| b/Å | 7.8852(9) | 11.6177(7) |
| c/Å | 13.027(2) | 33.308(9) |
| α° | 90 | 90 |
| β° | 90 | 93.45(2) |
| γ° | 90 | 90 |
| V/Å³ | 1799.1(4) | 1936.7(6) |
| Z | 4 | 4 |
| ρ(calc) mg/cm³ | 1.257 | 1.278 |
| μ/mm⁻¹ | 0.304 | 0.392 |
| F(000) | 728.0 | 792.0 |
| Crystal size/mm³ | 0.54 × 0.26 × 0.22 | 0.71 × 0.30 × 0.28 |
| 2θ range for data collection | 4.66 to 62.82° | 6.03 to 62.04° |
| Index ranges | -25 ≤ h ≤ 22, -11 ≤ k ≤ 10, -18 ≤ l ≤ 17 | -25 ≤ h ≤ 22, -16 ≤ k ≤ 15, -36 ≤ l ≤ 29 |
| Reflections collected | 9025 | 4900 |
| Independent reflections | 2712[R_{int} = 0.0737, R_{wp} = 0.0479] | 3079[R_{int} = 0.0198, R_{wp} = 0.0331] |
| Data/restraints/parameters | 2712/0/126 | 3079/0/212 |
| Goodness-of-fit on F² | 1.057 | 1.050 |
| Final R indexes [I>2σ(I)] | R₁ = 0.0570, wR₂ = 0.1524 | R₁ = 0.0744, wR₂ = 0.1750 |
| Final R indexes [all data] | R₁ = 0.0898, wR₂ = 0.1934 | R₁ = 0.1034, wR₂ = 0.1943 |
| Largest diff. peak/hole / e Å⁻³ | 0.32/-0.29 | 0.40/-0.22 |

*Figure 3. Reagents and conditions: a) PdCl₂, CH₃CN, 20 °C, 3h; b) CuCl₂, CH₃CN, 60 °C, 3h.*
Parameters of acute toxicity
To get reliable results acute toxicity was determined with Litchfield and Wilcoxon method modified by Prozorovskiy. As a result of determining the comparative evaluation of acute toxicity in albino mice after intraperitoneal injection and oral administration, it was established that compounds 1a, 2a and 3 are assigned to the group of virtually non-toxic compounds (1001 – 3000 mg/kg) according to the Sidorov classification (Table 2). After oral administration, LD50 value does not differ essentially from those of intraperitoneal administration. Thus, structure-activity relationship shows that sulphanyl bis-diketone 1a is less toxical compound (Sidorov classification, non-toxic group). It is not trivial fact that Pd(II) cis-chelate S,S-complex 3 also refers to group being not toxic. As seen from the Table 1, Cu(I) cis-chelate S,S-complex 4 is more toxical compound (Sidorov classification, low-toxic group). For this reason, compound 4 is not promising to treat liver diseases.

The model of acute hepatitis
Screening studies of compounds 1a and 3 were carried out on a model of acute toxicity in vivo. Simulation of animal-related processes was carried out with the principles of bioethics. The animals received single dose of CCl4 0.2 mL/kg intraperitoneally as a 50 percent solution of olive oil. The compound was administered intraperitoneally in a dose of 25 mg/kg 1 hour before the injection of CCl4. As control was used SAM (ademetionine), a pharmacologically known hepatoprotective drug in a dose of 25 mg/kg. The control group received 0.2 mL of saline solution (Table 3). Thus, compound 3 in dose of 25 mg/kg has a strong antitoxic effect on the model of acute intoxication of CCl4. In other words the compound 3 in the dose of 25 mg/kg causes significant lowering of lethality from 50 percent to 0 percent, compared with untreated animals in control group. Biochemical analysis of blood were taken on the 10-th day of observation of acute hepatitis, caused with CCl4, to control the development of cytolitic syndrome and evaluate degree of liver injury. The complex 3 at dose 25 mg/kg resulted in a significant lowering (p<0.05) alanine aminotransferase by 70% comparing with untreated group of animals. At the same time the reference preparation ademetionine at the same dose resulted a significant lowering only by 53% (p<0.05) (Table 4). Compounds 1a and 2a are less active according to this indicator. A significant lowering of aspartate aminotransferase between control and experimental groups was not recorded. By the 10 day of observation in all groups with an acute hepatitis the level of conjugated bilirubin raised. Compared to the intact group of animals figures receiving compound 3 – by 20.2%, 2a – at 55.9%, 1a – 58.8%, SAM – 25.9% in the group with the drug 3 - less often.

Table 2. Acute toxicity study of derivatives of 1,2-bis[(pentane-2,4-dione-3-yl)methylsulfanyl]ethane

| Entry | Test compound LD50, mg/kg | LD50 mg/kg |
|-------|--------------------------|-----------|
| 1     | 1a                       | 1580.0    |
| 2     | 2a                       | 1050.0    |
| 3     | 3                        | 1240.0    |
| 4     | 4                        | 74.5      |
| 5     | 5                        | 4650.0    |

272 | Advanced Pharmaceutical Bulletin, 2018, 8(2), 267-275
Pd(II) Complex of sulfanyl-isoxazole as a hepatoprotective agent

Histological examination

Staining with hematoxylin, eosin, by standard methods on histological complex MICROM. Histological activity index (HAI) was defined. Evaluation System indicators protein dystrophy, inflammatory infiltration, hyaline drop dystrophy - a 4-point scale.

Administering CCl₄ without treatment led to gross structural changes in the form of a large-drop dystrophy of hepatocytes, lymphohistiocytic infiltration of the liver structure.

Compounds pretreatment and heptral at dose 25 mg/kg led to less expression of morphological changes of liver structures: reduction of inflammatory infiltration, necrosis of hepatocytes, hepatocyte degeneration reduction degree.

Semi-quantitative method for assessing the degree of activity of pathological processes in the liver showed:

1) Significant reduction in HAI compared with results of control group was observed during therapy with Compound 3 and heptral. Compounds 1a and 2a had no significant digits.

2) The degree of fatty liver among white mice, treated with Compound 3 was minimal, hepatocytes with fatty inclusions are located only on the periphery of the hepatic lobule. Other animal groups 1a and 2a had moderate degree – 1/3 – 1/4 the length of the hepatic beams, heaths cirrhosis, liver tissue was sealed.

A new sensibly nontoxic (IV class) compound cis-S,S-dichlorido-1,6-(3,5-dimethylisoxazol-4-yl)-2,5-dithiahexane palladium(II) complex 3 with hepatoprotective activity in laboratory animals (white mice) at a dose of 25 mg/kg intraperitoneally on acute hepatitis model induced by carbon tetrachloride was discovered. Compound 3 exceled the reference preparation ademetionine (SAM) for indications:

a) animal survival (100%, SAM – 80%);

b) biochemical (ALT, AST, bilirubin direct) parameters;

c) histological (liver parenchyma lesions are minimal) parameters.

On the basis of biochemical tests (ALT, AST, bilirubin) and histological compounds displayed hepatoprotective activity which decreased in the number of 3 > 1a > 2a.

Conclusion

In summary, we have developed a two step effective synthesis of a,ω-bis(3,5-dimethylisoxazol-4-ylmethylsulfanyl)alkanes via the interaction between 2,4-pentandione, CH₂O, α,ω-diethils and next with hydroxyl amine. It was shown, that new Pd(II) and Cu(I) complexes are efficiently formed when using 1,2-bis(isoxazol-4-ylmethylsulfanyl)ethane as ligand. The in vivo method has demonstrated, that combinatorial row - 1,2-bis[(pentane-2,4-dione-3-yl)methylsulfanyl]ethane 1a, 1,2-bis[(sulfanyl)methyl(3,5-dimethylisoxazol-4-yl)]ethane 2a and its complex with PdCl₂ dichlorodi(3,5-dimethylisoxazol-4-yl)-1,2-dithiaethane palladium(II) 3 are virtually non-toxic and exhibit hepatoprotective activity. The leader among them is palladium(II) complex dichlorodi(3,5-dimethylisoxazol-4-yl)-1,2-dithiaethane 3, whose activity is comparable to SAM.

Acknowledgments

This work was partially financially supported by the Grant of the republic of Bashkortostan young scientists and youth research teams. The reported study was funded

---

Table 3. Effects of compounds 1a, 2a, 3 and SAM 5 survival of white mice with acute toxic hepatitis

| Administering compounds | Number of animals in groups | Survival rate on the 10 day of observation, % |
|-------------------------|-----------------------------|---------------------------------------------|
| Saline solution 0.2 mL/kg (intact group) | 10 | 100 |
| CCl₄ 0.2 mL/kg (control group) | 10 | 50 |
| SAM 25 mg/kg + CCl₄ 0.2 mg/kg | 10 | 80 |
| 1a 25 mg/kg + CCl₄ 0.2 mg/kg | 10 | 70 |
| 2a 25 mg/kg + CCl₄ 0.2 mg/kg | 10 | 60 |
| 3 25 mg/kg + CCl₄ 0.2 mg/kg | 10 | 100 |

Table 4. Effects of compounds 1a, 2a, 3 and SAM on indicators AST, ALT, and direct bilirubin serum white mice with acute toxic hepatitis

| Groups of animals | ALT, mc mol/mL/h | AST, mc mol/mL/h | Bilirubin direct serum, mc mol/L |
|-------------------|------------------|------------------|-------------------------------|
| Control intact    | 1.49 ± 0.22      | 1.09 ± 0.07      | 8.3 ± 2.2                     |
| Control (CCl₄)    | 4.72 ± 0.57*     | 1.71 ± 0.14*     | 19.5 ± 5.1*                   |
| SAM + CCl₄       | 2.21 ± 0.21      | 0.92 ± 0.06      | 11.2 ± 3.2                    |
| 3 + CCl₄         | 1.51 ± 0.31**    | 0.98 ± 0.02**    | 10.4 ± 2.6**                  |
| 2a + CCl₄        | 2.32 ± 0.48**    | 0.94 ± 0.06**    | 20.1 ± 4.5                    |
| 1a + CCl₄        | 2.68 ± 0.64      | 0.93 ± 0.07      | 18.8 ± 5.1                    |

Note: * - significant differences between indicators of intact animals, ** - significant differences from that of group CCl₄
by Russian Foundation for Basic Research and Academy of Sciences of the Republic of Bashkortostan according to the research project № 17-43-020299 p_a and project part 4.6007.2017/8.9. Structural studies of the compounds obtained were performed using unique equipment in "Agidel" collective usage centre (state assignment AAAA-A17-117012610060-7).

Ethical Issues
The study was carried out under ethical principles. Permission from the Local Ethics Committee of Bashkir state medical university is presented in supporting information.

Conflict of Interest
The authors declare that they have no conflict of interest.

References
1. Jaouen G, Salmain M, Vessieres A. Bioorganicmetallics: Biomolecules, labeling, medicine. Weinheim: Willey-VCH Verlag GmbH and KGaA; 2006.
2. Crisponi G, Nurchi VM, Lachowicz JI, Crespo-Osredkar J, Sustar N. Copper and zinc, biological role and significance of copper/zinc imbalance. Pure Appl Chem 2007;79(12):2243-61. doi: 10.1351/pac200779122243
3. Coluccia M, Natille G. Trans-platinum complexes in cancer therapy. Anticancer Agents Med Chem 2007;7(1):111-23. doi: 10.2174/187152007779314080
4. Bano N, Najam R, Qazi F. Adverse cardiac manifestations of cisplatin - A review. Int J Pharm Sci Res 2013;18(1):80-5.
5. Yao X, Panichpaisal K, Kurtzman N, Nugent K. Cisplatin nephrotoxicity: A review. Am J Med Sci 2007;334(2):115-24. doi: 10.1097/MAJ.0b013e31812df1e
6. Prudhomme M. Advances in anticancer agents in medicinal chemistry. France: Bentham Science Publishers; 2013.
7. Wang, Guo Z. The role of sulfur in platinum anticancer chemotherapy. Anticancer Agents Med Chem 2007;7(1):19-34. doi: 10.2174/187152007779314062
8. Kapdi AR, Fairlamb IJ. Anti-cancer palladium complexes: A focus on PdX2-L2, palladacycles and related complexes. Chem Soc Rev 2014;43(13):4751-77. doi: 10.1039/c4cs00063c
9. Amoke OA. Synthesis, characterization, in-vitro antibacterial and anticancer studies on some metal(II) complexes of (methylsulfanyll)chromenol Schiff base. Elixir Appl Chem 2011;39:4827-31.
10. Grigorieva AS, Drogovoz SM, Kirichek LM, Konahovich NF, Kuzmenko II, Mokhort NA, et al. Method of treating liver diseases of various origins. Patent RU 2035906, 1995.
11. Akhmetova VR, Akhmadiev NS, Starikova ZA, Tulyabaev AR, Mescheryakova ES, Ibragimov AG. Catalytic multicomponent thiomethylation of aliphatic 1,3-diketones as efficient one-pot synthesis of novel bis(1,3-diketone-2-ylmethylsulphanyl)alkanes. Tetrahedron 2015;71(40):7722-8. doi: 10.1016/j.tet.2015.07.055
12. Akhmetova VR, Akhmadiev NS, Meshcheryakova ES, Khalilov LM, Ibragimov AG. Multicomponent synthesis and biological activity of (sulfanylalkyl)-substituted azaheterocycles. Chem Heterocycl Compd 2014;50(5):742-51. doi: 10.1007/s10593-014-1529-9
13. Maksimov V, Zaynullin R, Akhmadiev N, Segura-Cенерос EP, Martinez Hernandez JL, Bikhulatova E, et al. Inhibitory effect of 4'-jethane-1,2-diybis(sulfandiylmethanediyl)bis(3,5-dimethyl-1H-pyrazole) and its derivatives on alpha-amylase activity. Med Chem Res 2016;25(7):1384-9. doi: 10.1007/s00044-016-1574-2
14. Akhmetova VR, Akhmadiev NS, Ibragimov AG. Catalytic multicomponent reactions of 1,3-dicarbonyl CH-acids with CH2O and S- and N-nucleophiles. Russ Chem Bull 2016;65(7):1653-66. doi: 10.1007/s11172-017-1495-3
15. Galenko AV, Khlebnikov AF, Novikov MS, Pakalnis VV, Rostovskii NV. Recent advances in isoxazole
Pd(II) Complex of sulfanyl-isoxazole as a hepatoprotective agent

Advanced Pharmaceutical Bulletin, 2018, 8(2), 267-275 | 275

25. Kumar KA, Jayaroopa P. Pyrazoles: synthetic strategies and their pharmaceutical applications—an overview. Int J Pharm Tech Res 2013;5(4):1473-86.

26. Pradeepkumar Y, Ruthu M, Madhusudhana chetty C, Prasanthi G, Jaya Sankar Reddy V. Pharmacological activities of isoxazole derivatives. J Global Trends Pharm Sci 2011;2(1):55-62.

27. Pinto A, Tamborini L, Cullia G, Conti P, De Micheli C. Inspired by Nature: The 3-Halo-4,5-dihydroisoxazole Moiety as a Novel Molecular Warhead for the Design of Covalent Inhibitors. ChemMedChem 2016;11(1):10-4. doi: 10.1002/cmdc.201500496

28. Urdaneta N, Landaeta VR, Rodríguez-Lugo RE, Díaz C, Santiso-Quinones G, Quiroga J, et al. Synthesis and characterization of Cu(I) and Zn(II) complexes with new sulfur-bearing isoxazole- or pyrazole-based ligands. Inorg Chem Commun 2015;55:43-7. doi: 10.1016/j.inoche.2015.03.007

29. Munsey MS, Natale NR. The coordination chemistry of isoxazoles. Coord Chem Rev 1991;109(2):251-81. doi: 10.1016/0010-8545(91)80019-A

30. Sheldrick GM. A short history of SHELX. Acta Crystallogr A 2008;64(Pt 1):112-22. doi: 10.1107/S0108767307043930

31. Dolomanov OV, Bourhis LJ, Gildea RJ, Howard JAK, Puschmann H. OLEX2: a complete structure solution, refinement and analysis program. J Appl Cryst 2009;42:339-41. doi: 10.1107/S0021889808042726

32. Mironov AN. A guide to preclinical drug research. Moscow: Grif and K; 2012.

33. Singh A, Bhat TK, Sharma OP. Clinical biochemistry of hepatotoxicity. J Clin Toxicol 2011;51(2):181. doi: 10.1002/jctd.201100112

34. Pandit A, Sachdeva T, Bafna P. Drug-induced hepatotoxicity: a review. J Appl Pharm Sci 2012;2(5):233-43.

35. Bigoniya P, Singh CS, Shukla A. A comprehensive review of different liver toxicants used in experimental pharmacology. Int J Pharm Sci Drug Res 2009;1(3):124-35.

36. Lu SC. S-Adenosylmethionine. Int J Biochem Cell Biol 2000;32(4):391-5. doi: 10.1016/s1357-2725(99)00139-9

37. Anstee QM, Day CP. S-Adenosylmethionine (SAMe) therapy in liver disease: a review of current evidence and clinical utility. J Hepatol 2012;57(5):1097-109. doi: 10.1016/j.jhep.2012.04.041

38. Kucheryavy YuA, Morozov SV. Hepatoprotectors: rational aspects of the application. Moscow: Fort Drum(Russia); 2012.

39. Okovity SV, Sukhanov DS, Petrov AYu, Romantssov MG. Hepatotropic medicines: current status. Ther Arch 2012;84(2):62-8.

40. Berezovskaya IV. Classification of substances with respect to acute toxicity for parenteral administration. Pharm Chem J 2003;37(3):139-41. doi: 10.1023/A:1024586630954

41. Center SA. S-adenosyl-methionine (SAMe) an antioxidant and anti-inflammatory nutraceutical. 18th ed. Seattle, WA: ACVIM; 2000.