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Synthesis and Primary Evaluation of the Hepatoprotective Properties of Novel Pyrimidine Derivatives

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Abstract—Based on the active ingredient of the drug Ximedon (1,2-dihydro-4,6-dimethyl-1-*N*-(2-hydroxyethyl)pyrimidone-2, referred below to as pyrimidine (**I**), novel derivatives containing biogenic acids: succinic, L-ascorbic, *para*-aminobenzoic, nicotinic, and L-2-amino-4-(methylthio)butanoic (L-methionine) acids have been synthesized. The parameters of acute toxicity (LD_{50}) have been studied. The antitoxic effect of the compounds upon the injury by the hepatotropic poison carbon tetrachloride has been examined as the primary evaluation of their hepatoprotective properties. It has been found that, according to toxicological safety, the compounds synthesized belong to classes III and IV (moderately and little toxic compounds). The conjugates of pyrimidine (**I**) with ascorbic acid and methionine (LD_{50} more than 5400 mg/kg) are least toxic. Pyrimidine (**I**) and its derivatives possess the antitoxic activity upon acute poisoning with carbon tetrachloride; the combined injection of carbon tetrachloride with pyrimidine (**I**) or its derivatives leads to an increase in the survival of animals and the normalization of the integral functional parameters, weight and body temperature, which decrease upon toxic injury. In addition, pyrimidine (**I**) and some of its derivatives (conjugates with L-ascorbic, succinic, *para*-aminobenzoic, and nicotinic acids) decrease the weight coefficients of the liver and kidneys (the organ-to-body-weight ratio) and the activity of transaminases, the markers of hepatic cytolysis, which increase upon toxic injury with carbon tetrachloride. The area of the pathological injury of the liver by steatosis and necrosis decreases by the action of pyrimidine (**I**) and its novel derivatives (conjugates with L-ascorbic, succinic, and nicotinic acids) two to three times. Advantages of pyrimidine (**I**) and its novel derivatives over the hepatoprotective drug Thiotriazolin have been revealed.

Keywords: pyrimidines, Ximedon, hepatoprotectors, liver diseases, toxic hepatitis

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INTRODUCTION

According to the data of the World Health Organization, more than two billion people in the world suffer from liver diseases. This situation requires an increasingly frequent prescription of hepatoprotectors whose main function is the prophylaxis and treatment of liver cells for injuries induced by hepatotoxins. In this connection, a search for hepatoprotectors is one of the priority problems of national public health.

Pyrimidine derivatives attract attention as potential hepatoprotectors owing to their capacity to stimulate tissue regeneration. Among pyrimidine derivatives, the drug oxymethyluracil (2-methyl-4-amino-6-oxypyrimidine) has been found to exhibit hepatoprotective properties [1]. We have shown earlier that the pyrimidine derivative 1,2-dihydro-4,6-dimethyl-1-*N*-(2-hydroxyethyl-pyrimid-2-on (compound (**I**) in Fig. 1), which is an active ingredient of the medicine Ximedon, and its L-ascorbate (compound (**III**) in Fig. 1) hold promise; they increase the adaptation reserves of the organism under stress conditions of high physical loads in the "forced swimming" test [2] and stimulate the liver regeneration after toxic injury by carbon tetrachloride [3, 4]. In addition, it has been shown that pyrimidine derivatives based on Ximedon possess a neuroprotective activity by beneficially affecting the regeneration of spinal cortex tissue after traumas [5, 6].

The aim of the present work was to study the hepatoprotective effect of novel pyrimidine derivatives of the active ingredient (**I**) of Ximedon, its salt-like conjugates with biogenic acids: succinic, L-ascorbic, *para*-aminobenzoic, nicotinic, and L-2-amino-4-(methylthio)butanoic (L-methionine) acids (com-

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Abbreviations: LD_{50}, a dose that causes the death of 50% of animals; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

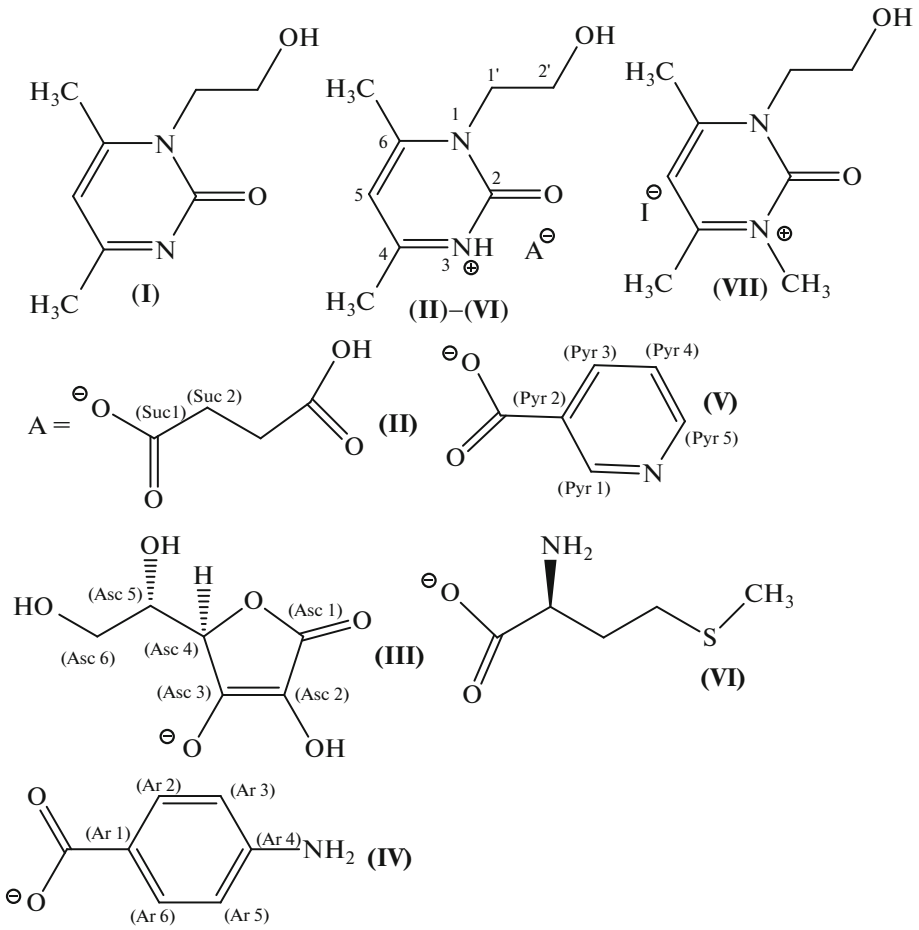

Fig. 1. Ximedon and its derivatives (**II**)–(**VII**). The numbering of the atoms of the compounds is indicated.

pounds (**II**)–(**VI**), respectively, on Fig. 1). In addition, we examined compound (**VII**) in which the N atom of the pyrimidine ring is alkylated by the methyl group (Fig. 1). We believe that the conjugation of pyrimidine derivative (**I**) with biogenic acids would enhance its hepatoprotective properties and make it possible to design novel drugs for liver protection. Here, we made a primary evaluation of the hepatoprotective properties (antitoxic effect of the substances upon toxic injury by carbon tetroxide) of compounds (**II**)–(**VII**) in comparison with pyrimidine (**I**) and the hepatoprotective drug Thiotriazolin.

RESULTS AND DISCUSSION

Conjugates (**II**)–(**VI**) (Fig. 1) were obtained by heating a mixture of pyrimidine (**I**) and the corresponding acid in alcohol or water. The structures of compounds (**II**)–(**VI**) were confirmed by homo- and heterocorrelation NMR spectroscopy (2D ^{1}H-^{1}H-COSY, 2D ^{1}H-^{13}C-HSQC/HMBC, ^{13}C, DEPT, and IR spectroscopy), and the composition was determined using the elemental analysis data. The ^{1}H NMR and ^{13}C NMR spectra of compounds (**II**)–(**VI**) contain resonance signals of both the pyrimidine fragment and the fragment of the acid. The IR spectra of compounds (**II**)–(**VI**) are almost additive to the spectra of pyrimidine (**I**) and the corresponding acid. In addition, an X-ray diffraction study of some of the resulting conjugates was performed, and the results will be reported elsewhere.

To assess the prospects for the use of the novel pyrimidine (**I**) derivatives as hepatoprotectors, a primary screening was carried out by the method described in [7]. The first stage of selecting the compounds for the tests of hepatoprotective properties involves the determination of acute toxicity parameters. In the present work, acute toxicity was examined on nonlinear white mice weighing 18–25 g. A test compound in an aqueous solution was injected intraperitoneally (i.p.) to mice in four to five increasing doses; each group contained four to six animals. The semilethal dose (LD_{50}) was calculated using the program "R", version 2.13.0 (2011-04-13). Based on LD_{50} values (Table 1), most compounds can be assigned, according to [8], to III or IV classes of toxicity; and compounds (**III**) and (**VI**), to class VI. Thus, based on acute toxicity values and in accordance with [7], all

compounds synthesized can be potentially interesting for studies of the hepatoprotective activity.

The hepatoprotective properties of the pyrimidine derivatives were tested on nonlinear white mature rats of both sexes (weight of males 250–350 g, weight of females 200–300 g). The total number of animals in each group was 10 or 11. For the purpose of prophylaxis, the compounds being tested were injected i.p. to animals for 11 days at doses of 1/300 of LD_{50} (Table 1). The hepatoprotector Thiotriazolin was used as a reference substance. The effect of a substance at these doses on the clinical state of healthy animals was assessed from changes in the body weight over an 11-day period of the injection of the compound until the induction of toxic injury. Beginning from day 12, the toxic injury of the liver was induced for three days together with the injection of the test compounds as described in [7].

The results of the experiments showed that, in the control group, which did not receive therapy, the survival of animals after the poisoning with CCl_4 was 84% (16 of 19 animals). The highest survival (100%) after the poisoning was noted in groups in which compound (**V**), pyrimidine (**I**), and Thiotriazolin were injected. In groups of animals injected with compounds (**II**) and (**IV**), the survival was lower (90–92%) but nevertheless higher than in the control group (Table 2). Because the changes in the survival of animals were statistically insignificant, as compared with the control group (criterion χ^2), we can say that there was only a tendency for the manifestation of this antitoxic effect upon the poisoning with carbon tetrachloride. The survival in the other groups did not differ from the control.

The examination of the body weight of animals during the 11-day course of injections of the test compounds showed that the compounds at a dose of 1/300 of LD_{50} had no adverse effect on the dynamics of changes in body weight, and the clinical state of animals was normal. In the group of animals injected with compound (**VI**) (dose 20 mg/kg, males), a statistically significant increase in the average body weight of rats by 11.5 ± 6.6 g ($p < 0.05$) was observed by day 8 of injection. In this case, the body weight exceeding the initial values by 14.2 ± 7.3 g was retained up to the third poisoning with CCl_4. A similar effect was observed with compound (**VII**). By day 4 of its injection, the body weight of animals increased by 7.4 ± 3.6 g ($p < 0.05$). Higher body weight values, which exceeded the initial values by 12.4 ± 5.9 g, were observed in this group even after the second administration of CCl_4. In the other experimental groups (injection with Thiotriazolin, pyrimidine (**I**), compounds (**II**)–(**V**), and (**VII**)) and in the control, no statistically significant changes in body weight within 11 days of the injection of the preparations was found ($p > 0.05$).

In males of the control group, the body weight after the second and third injection of CCl_4 decreased by 24.1 ± 10.9 g. The differences from the initial body weight values were statistically significant ($p < 0.05$). In females, no statistically significant changes in body weight were revealed after the poisoning with CCl_4.

Table 1. Parameters of acute toxicity (LD_{50}) of compounds under study and the doses used for rats

Compound	LD_{50}, mice, i.p., mg/kg	Dose, 1/300 of LD_{50}, mg/kg
Thiotriazolin	5890	20.0
(**I**) Ximedon	More than 6500	20.0
(**II**)	827 (616–1242)	3.0
(**III**)	5427 (3690–7023)	20.0
(**IV**)	1686 (1409–1943)	6.0
(**V**)	1909 (1804–2172)	6.0
(**VI**)	5459 (4313–7264)	20.0
(**VII**)	1800	6.0

The retention of the body weight of animals, the absence of differences from the initial values, and a reduction in weight loss served in our study as one of the criteria of the antitoxic effect of the compounds upon toxic injury with CCl_4.

In most experimental groups, as well as in the control, no statistically significant changes in body weight of females were observed after the administration of CCl_4; therefore, the effect of the test compounds on this parameter was analyzed only on males.

After the injection of Thiotriazolin, Ximedon, and the novel pyrimidine derivatives (**III**), (**V**), (**VI**), and (**VII**), the average body weight of males under the toxic effect of CCl_4 remained at the initial level. In animals injected with compounds (**II**) and (**IV**), the body weight after the second and third administration of CCl_4 decreased. In this case, the decrease in body weight after the injection of (**II**) was observed not only in males but also in females; the effect of compound (**IV**) was noted even after the first injection of CCl_4. The reduction in body weight of males in both groups was less than in the control (Table 2).

The examination of body temperature during the combined administration of the test compounds and CCl_4 revealed a progressive decrease in the body temperature of animals in the control group from 38.6°C (prior to poisoning) to 33.2°C (on the next day after the last administration of CCl_4). The most substantial decrease in body temperature was characteristic for the terminal stage of poisoning, which preceded the lethal outcome (Table 2). Therefore, a decrease in body temperature of animals was taken as one of the criteria in the evaluation of the toxic effect of CCl_4. A decrease in the body temperature decline by the action of test compounds was taken, correspondingly, as a criterion in the evaluation of their antitoxic effect.

Table 2. Analysis of the antitoxic effect of pyrimidine derivatives relative to the toxic injury

Substances used and treatment	Survival		Body temperature during the injection of CCl_4, °C				Decrease in body weight after the injection of CCl_4, g	
	%	number of animals	initial	after the 1st injection	after the 2nd injection	after the 3rd injection	males	females
Intact (no treatment)	100	15/15	38.84 ± 0.07	38.40 ± 0.21 $p < 0.001$	38.77 ± 0.32 $p < 0.001$	38.26 ± 0.20 $p < 0.001$	–	–
Control: H_2O + CCl_4	84.2	16/19	38.59 ± 0.14	36.09 ± 0.38	35.35 ± 0.23	33.19 ± 0.17	24.1 ± 10.9*	**Not found ($p > 0.05$)**
Thiotriazolin + CCl_4	**100**	**11/11**	38.66 ± 0.16	35.73 ± 0.43	35.27 ± 0.28	33.55 ± 0.34	**Not found ($p > 0.05$)**	**Not found($p > 0.05$)**
Pyrimidine (**I**) (Ximedon) + CCl_4	**100**	**11/11**	38.10 ± 0.38	36.59 ± 0.32	35.87 ± 0.28	**34.65 ± 0.41** $p < 0.001$	**Not found ($p > 0.05$)**	**Not found ($p > 0.05$)**
(**II**) 3.0 mg/kg i.p. + CCl_4	**92.3**	**12/13**	38.43 ± 0.16	35.72 ± 0.38	35.66 ± 0.37	**33.85 ± 0.24** $p < 0.05$	19.0 ± 2.6*	12.0 ± 5.7*
(**III**) 20.0 mg/kg i.p. + CCl_4	81.8	9/11	38.32 ± 0.26	36.17 ± 0.44	35.64 ± 0.31	**34.76 ± 0.47** $p < 0.001$	**Not found ($p > 0.05$)**	**Not found ($p > 0.05$)**
(**IV**) 6.0 mg/kg i.p. + CCl_4	**90.9**	**10/11**	38.48 ± 0.16	36.11 ± 0.34	35.59 ± 0.40	**33.86 ± 0.39**	16.0 ± 3.2*	**Not found ($p > 0.05$)**
(**V**) 6.0 mg/kg i.p. + CCl_4	**100**	**11/11**	38.24 ± 0.27	36.42 ± 0.27	35.56 ± 0.27	**34.61 ± 0.41** $p < 0.01$	**Not found ($p > 0.05$)**	**Not found ($p > 0.05$)**
(**VI**) 20.0 mg/kg i.p. + CCl_4	72.7	8/11	38.27 ± 0.15	36.28 ± 0.19	35.45 ± 0.25	33.66 ± 0.26	**Not found ($p > 0.05$)**	–
(**VII**) 6.0 mg/kg i.p. + CCl_4	81.8	9/11	37.80±0.27**	35.95 ± 0.26	35.20 ± 0.40	**34.70 ± 0.29** $p < 0.001$	**Not found ($p > 0.05$)**	–

Average values and the standard errors of the means ($M \pm m$) are given; values indicating an increase in the efficiency of the compounds as compared with the control with the use of CCl_4 are shown by boldface type. The statistical significance of temperature differences between the data sets for control and experimental groups was determined by the Student's t-test for independent samplings from integrated data for males and females.

* A decrease in the body weight of rats during poisoning with CCl_4 compared with the initial weight at the beginning of the experiment and the statistical significance of these differences at $p < 0.05$ calculated using the nonparametric Wilcoxon test are given.

The combined administration of the test compounds and CCl_4 caused a decrease in body temperature of animals in all groups; after the first and second injections of CCl_4, no significant differences between the groups were found. After the third injection of CCl_4, the decrease in body temperature in the control group was most clearly pronounced; in this period, the differences in the dynamics of temperature changes between experimental and control groups were most significant. The highest body temperatures after the third administration of CCl_4 (34.76 ± 0.47°C) were in the group of animals injected with compound (**III**) (differences from the control are statistically significant at $p < 0.001$). Among the other compounds, the following substances had a statistically significant beneficial effect on this parameter (in the order of decreasing effect): (**VII**), (**I**), and (**V**) (Table 2). Statistically significant differences in body temperature on day 3 of the CCl_4 injection ($p < 0.05$, the *t*-test for independent data samplings) compared to the control group were also revealed in the group of animals injected with compound (**II**). The body temperatures in this group were 33.85 ± 0.24°C. In the group of animals injected with compound (**IV**), the average temperature after the third injection of CCl_4 was at a similar level (33.86 ± 0.39°C); however, the differences from the control were statistically insignificant (Table 2).

The examination of the weight of the liver relative to the body weight showed that, by the action of CCl_4, the liver weight increased 1.4 times, and the weight of the right and left kidneys increased 1.6 times. The differences of these values from the reference values for intact animals were statistically significant at $p < 0.001$ (the Student's *t*-test). In groups of animals injected with the pyrimidine derivatives tested, the relative weight of the liver decreased compared with the control, indicating diminishing pathological changes and the normalization of the function of the organ (Table 3). Along with a decrease in the relative weight of the liver, some compounds induced a reduction in the relative weight of kidneys. These are compounds (**IV**), (**V**), (**VII**), pyrimidine (**I**), and Thiotriazolin. A comparison of the data on the relative weight of organs with the control group showed that the differences determined by the Student's *t*-test are statistically insignificant.

The study of the biochemical parameters in the control group of animals (without prophylactic treatment with test preparations) revealed that the level of the liver injury markers ALT and AST increased 7.4 and 7.7 times, respectively (Table 3). Compounds (**IV**), (**V**), and (**VII**) were active toward the markers of hepatic cytolysis; the level of ALT by the action of these compounds injected according to the scheme used decreased 1.5–2.4 times, and the level of AST decreased by 15–60% compared with the control group, indicating their hepatoprotective activity and their advantage over the reference preparation Thiotriazolin and the initial compound pyrimidine (**I**). A morphometric examination showed that the area of injury was 36.30 ± 2.71% in the control, 19.08 ± 5.09% after the injection of pyrimidine (**I**), 22.07 ± 2.53% after the injection of (**II**), 15.89 ± 2.16 after the injection of (**III**), 31.40 ± 7.11% after the injection of (**IV**), 24.03 ± 3.17% after the injection of (**V**), 38.14 ± 2.25% after the injection of (**VI**), 35.94 ± 6.78% after the injection of (**VII**), and 33.19 ± 3.51% after the injection of Thiotriazolin. The least area of liver injury, which statistically significantly differed from the control, was observed after the injection of pyrimidine (**I**) and compounds (**II**), (**III**), and (**V**). Compound (**III**) led to the most pronounced decrease in the injured area, which was 17% less compared with the effect of pyrimidine (**I**).

Thus, the primary screening of the novel pyrimidine derivatives showed that these compounds are of low toxicity; compounds (**III**) and (**VI**), the conjugates of pyrimidine (**I**) with ascorbic acid and methionine, have the highest LD_{50} values, which are comparable with that for the reference preparation Thiotriazolin.

Upon acute poisoning with CCl_4, pyrimidine (**I**) produced a marked antitoxic effect, which manifested itself in increased survival of animals and the normalization of the integral parameters of their clinical state: weight and body temperature. Pyrimidine (**I**) has an advantage in the magnitude of the effect over the medicinal drug Thiotriazolin. Among the novel pyrimidine derivatives, compound (**V**) exhibited the greatest antitoxic effect by three evaluation criteria, and compounds (**III**) and (**VII**), by the normalization of body weight and temperature.

In the groups of animals injected with the pyrimidine derivatives tested, the relative weight of the liver, which increased by the toxic action of CCl_4, was less than in the control group, and the effect was comparable with the action Thiotriazolin. Compounds (**IV**), (**III**), (VI) induced the greatest reduction in the relative liver weight. Along with a decrease in the relative liver weight, compounds (**IV**), (**V**), (VII), pyrimidine (**I**), and Thiotriazolin induced a decrease in the relative weight of the kidneys upon the toxic injury by CCl_4.

The results of the study suggest that further thorough research into the hepatoprotective properties of pyrimidine derivatives based on the active substance of the drug Ximedon and biogenic acids holds promise. Leading compounds have been identified that produce the most pronounced impact on the activity of transaminases, the markers of hepatic cytolysis: these are (**II**), (IV), and (**VII**); compounds that have the lowest toxicity and marked antitoxic activity upon acute poisoning with CCl_4: (**III**), and (**V**); and compounds decreasing the area of the injured liver tissue to the greatest extent: (**II**), (**III**), and (**V**).

Table 3. Effect of compounds and the poisoning with CCl_4 on the weight coefficients of the liver and kidneys and biochemical markers of hepatic cytolysis in rats

Substances used and treatment	Liver, % of body weight	Left kidney, % of body weight	Right kidney, % of body weight	ALT, U/L	AST, U/L	Area of liver injury, %
Intact (no treatment)	2.98 ± 0.14	0.30 ± 0.01	0.31 ± 0.01	39.9 (33.9–47.6)	85.4 (73.2–105.0)	0.00 ± 0.00
Control: H_2O + CCl_4	4.26 ± 0.15[1]	0.47 ± 0.02[1]	0.50 ± 0.02[1]	296.6[1] (178.9–652.8)	659.4[1] (421.4–1392.2)	36.30 ± 2.71[1]
Thiotriazolin 20.0 mg/kg i.p. + CCl_4	**3.96 ± 0.17**[1]	**0.44 ± 0.02**[1]	**0.46 ± 0.02**[1]	590.9[1] (151.3–879.9)	715.5[1] (449.9–1807.0)	33.19 ± 3.51[1]
Pyrimidine (**I**) (Ximedon) 20.0 mg/kg i.p. + CCl_4	**4.01 ± 0.16**[1]	**0.44 ± 0.02**[1]	**0.46 ± 0.02**[1]	591.2[1] (334.0–1522.5)	1572.4[1] (669.2–2187.0)	**19.08 ± 5.09**[1,2]
(**II**) 3.0 mg/kg i.p. + CCl_4	**3.99 ± 0.15**[1]	0.47 ± 0.02[1]	0.48 ± 0.02[1]	**192.8**[1] **(120.1–307.1)**	**519.9**[1] **(417.9–709.6)**	**22.07 ± 2.53**[1,2]
(**III**) 20.0 mg/kg i.p. + CCl_4	**3.88 ± 0.22**[1]	0.46 ± 0.04[1]	0.47 ± 0.04[1]	1189.3[1] (227.2–1993.0)	755.4[1] (592.8–1389.1)	**15.89 ± 2.16**[1,2]
(**IV**) 6.0 mg/kg i.p. + CCl_4	**3.75 ± 0.21**[1]	**0.42 ± 0.02**[1]	**0.44 ± 0.02**[1]	**122.3**[1, 2] **(110.4–308.6)**	**564.5**[1] **(430.5–1388.3)**	31.40 ± 7.11[1]
(**V**) 6.0 mg/kg i.p. + CCl_4	4.20 ± 0.19[1]	**0.43 ± 0.02**[1]	**0.43 ± 0.02**[1]	421.1[1] (168.4–884.1)	772.8[1] (691.9–1038.3)	**24.03 ± 3.17**[1,2]
(**VI**) 20.0 mg/kg i.p. + CCl_4	**3.81 ± 0.24**[1]	0.47 ± 0.05[1]	0.49 ± 0.05[1]	254 (159–614.3)	3654[1, 2] (195.8–4914)	38.14 ± 2.25[1]
(**VII**) 6.0 mg/kg i.p. + CCl_4	**3.99 ± 0.19**[1]	0.48 ± 0.07[1]	**0.43 ± 0.**03[1]	**184**[1] **(108.8–985.5)**	**393**[1, 2] **(35.5–393)**	35.94 ± 6.78[1]

For weight coefficients of the organs, the average values and the standard error of the means ($M \pm m$) are given; for biochemical parameters, a median is given (25–75 percentile). Values that were improved most distinctly as compared with the control group are shown in boldface type.

[1]Differences from the group of intact control are statistically significant at $p < 0.05$.

[2]Differences from the control group are statistically significant at $p < 0.05$. The differences in the weight coefficients for organs were determined using the *t*-test; the differences in the biochemical parameters of the area of liver injury were determined using the Mann–Whitney test.

EXPERIMENTAL

1,2-Dihydro-4,6-dimethyl-1-(2-hydroxyethyl)-pyrimidone-2 (Ximedon, (**I**)) was synthesized from 1,2-dihydro-4,6-dimethyl-pyrimid-2-one and 2-chloroethanol by the conventional method [9]. 1-(2-Hydroxyethyl)-1,2-dihydro-3,4,6-trimethyl-2-oxopyrimidinium iodide (**VII**) was also synthesized by the conventional technique [10]. Succinic acid and L-methionine were purchased from Acros Organics (United States); *para*-aminobenzoic and nicotinic acids were from Fisher Chemicals (United States); and L-ascorbic acid was from OAO Tatkhimfarmpreparaty (Russia).

1D and 2D NMR experiments (^1H, ^{13}C, δ, ppm; *J*, Hz) with compounds (**II**)–(**VI**) were carried out on an AVANCE-500 Fourier spectrometer (Bruker) at a working frequency of 500.13 MHz (^1H) and 125.77 MHz (^{13}C) in D_2O at 30°C with tetramethylsilane as an external standard. IR spectra of the compounds (ν_{max}, cm^{-1}) were recorded in KBr tablets on a Vector 22 Fourier spectrometer (Bruker) under standard conditions in the region of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. The elemental analysis was carried out on a C,H,N-analyzer. The melting temperatures of the compounds were measured on a Stuart SMP10 apparatus.

A general method for the synthesis of conjugates (II)–(VI). Equimolar amounts of pyrimidine (**I**) and the corresponding acid were dissolved in alcohol (methanol or ethanol). The mixture was stirred for 8 h at 40–50°C and cooled, and the precipitate was fil-

tered. The sediment was ground in acetone, the solvent was decanted, and the residue was dried in vacuo.

1,2-Dihydro-4,6-dimethyl-1-(2-hydroxyethyl)-pyrimid-2-one succinate (II) was obtained in ethanol (40 mL) from pyrimidine (**I**) (5.05 g, 30.0 mmol) and succinic acid (3.51 g, 30.0 mmol). The yield of conjugate (**II**): 8.40 g (98%); mp 96–97°C with decomposition; IR: 3420, 2953, 2693, 1761, 1671, 1639, 1601, 1428, 1142, 1110, 1038, 864; ^{1}H NMR: 6.60 (s, 1H, H5), 4.18 (t, 2H, H1', *J* 5.3), 3.86 (t, 2H, H2', *J* 5.3), 2.55 (s, 4H, H^{Suc2}), 2.53 (s, 3H, H6), 2.48 (s, 3H, H4); ^{13}C NMR {^{1}H}: 177.6 (C^{suc1}), 172.2 (C4), 165.1 (C6), 153.8 (C2), 108.03 (C5), 57.7 (C2'), 48.11 (C1'), 29.4 (C^{suc2}), 20.7 (C4), 19.6 (C6). Found, %: C 50.21; H 6.27; N 9.80. $C_{12}H_{18}N_2O_6$. Calculated, %: C 50.35; H 6.34; N 9.79.

1,2-Dihydro-4,6-dimethyl-1-(2-hydroxyethyl)-pyrimid-2-one L-ascorbate (III) was obtained in methanol (80 mL) from pyrimidine (**I**) (10 g, 60.0 mmol) and L-ascorbic acid (10.48 g, 60.0 mmol). Yield: 17.70 g (86%); mp 117–118°C; IR: 3430, 2990, 2653, 1729, 1695, 1420, 1311, 920, 638; ^{1}H NMR: 6.61 (s, 1H, H5), 4.70 (m, 1H, H^{Asc4}), 4.20 (t, 2H, CH1', *J* 5.3), 4.00 (t, 1H, CH^{Asc5}, *J* 6.1), 3.87 (t, 2H, H2', *J* 5.3), 3.70 (d, 1H, H^{Asc6}, *J* 6.1), 2.56 (s, 3H, H6), 2.39 (s, 3H, H4); ^{13}C NMR {^{1}H}: 174.6 (C^{Asc1}), 172.25 (C4), 164.98 (C6), 163.72 (C^{Asc2}), 154.0 (C2), 115.32 (C^{Asc2}), 108.1 (C5), 76.7 (C^{Asc4}), 68.72 (C^{Asc5}), 61.8 (C^{Asc6}), 57.8 (C2'), 48.1 (C1'), 20.7 (C4$\underline{C}H_3$), 19.5 (C6$\underline{C}H_3$). Found, %: C 49.01; H 6.00; N 7.98. $C_{14}H_{20}N_2O_8$. Calculated, %: C 48.85; H 5.85; N 8.14.

1,2-Dihydro-4,6-dimethyl-1-(2-hydroxyethyl)-pyrimid-2-one *para*-aminobenzoate (IV) was obtained in methanol (50 mL) from pyrimidine (**I**) (5 g, 29.8 mmol) and *para*-aminobenzoic acid (4.08 g, 29.8mmol). Yield: 9.0 g (89%); mp 113–114°C; IR: 3461, 3364, 2675, 1664, 1625, 1442, 1423, 1313, 1292, 1174, 843, 772; ^{1}H NMR: 7.65 (d, 2H, H^{Ar2}, *J* 8.6), 6.70 (d, 2H, H^{Ar3}, *J* 8.6), 6.40 (s, 1H, H5), 4.06 (t, 2H, H1', *J* 5.4), 3.81 (t, 2H, H2', *J* 5.4), 2.39 (s, 3H, H6), 2.26 (s, 3H, H4); ^{13}C NMR {^{1}H}: 173.91 (C4), 171.35 (COO), 161.98 (C6), 156.10 (C2), 150.36 (C^{Ar4}), 130.92 (C^{Ar2}), 120.53 (C^{Ar1}), 114.40 (C^{Ar3}), 108.13 (C5), 57.86 (C2'), 47.66 (C1'), 21.79 (C4$\underline{C}H_3$), 19.29 (C6$\underline{C}H_3$). Found, %: C, 59.15; H, 6.34; N, 13.64. $C_{15}H_{19}N_3O_4$. Calculated, %: C, 59.01; H, 6.27; N, 13.76.

1,2-Dihydro-4,6-dimethyl-1-(2-hydroxyethyl)-pyrimid-2-one nicotinate (V) was obtained in methanol (100 mL) from pyrimidine (**I**) (5.05 g, 30.0 mmol) and nicotinic acid (3.69 g, 30.69 mmol). Yield: 7.85 g (89%); mp 138–140°C; IR: 3409, 3071, 2456, 1714, 1651, 1602, 1545, 1415, 1323, 1088, 1038, 775, 748; ^{1}H NMR: 8.98 (s, 1H, H^{Pyr1}), 8.70 (d, 1H, H^{Pyr5}, *J* 5.5), 8.63 (d, 1H, H^{Pyr3}, *J* 8.0), 7.85 (dd, 1H, H^{Pyr4}, *J* 8.0, 5.5), 6.52 (s, 1H, H5), 4.14 (t, 2H, H1', *J* 5.4), 3.84 (t, 2H, H2', *J* 5.4), 2.50 (s, 3H, H6), 2.31 (s, 3H, CH4); ^{13}C NMR {^{1}H}: 173.1 (C4), 168.9 ($C^{Pyr2}\underline{C}OO$), 163.4 (C6), 155.1 (C2), 144.5 (C^{Pyr5}), 143.8 (C^{Pyr1}), 143.0 (C^{Pyr3}), 134.2 (C^{Pyr2}), 125.6 (C^{Pyr4}), 108.1 (C5), 57.8 (C2'), 47. 9 (C1'), 21.2 (C4$\underline{C}H_3$), 19.4 (C6$\underline{C}H_3$). Found, %: C, 57.35; H, 6.30; N, 14.54. $C_{12}H_{18}N_3O_4$. Calculated, %: C, 57.52; H, 6.21; N, 14.38.

1,2-Dihydro-4,6-dimethyl-1-(2-hydroxyethyl)-pyrimid-2-one L-methionate (VI) was obtained in water (100 mL) from pyrimidine (**I**) (5.64 g, 33.6 mmol) and L-2-amino-4-(methylthio)butanoic acid (5.0 g, 33.6 mmol). Yield: 9.54 g (90%); mp 140–145°C with decomposition; IR: 3410, 2917, 2605, 1651, 1606, 1512, 1410, 1348, 1244, 1088, 1022, 794; ^{1}H NMR: 6.45 (s, 1H, H5), 4.11 (t, 2H, H1', *J* 5.2), 3.83 (t, 2H, H2', *J* 5.2), 3.80 (m, 1H, H^{Mt2}), 2.58 (t, 2H, H^{Mt4}, *J* 7.6), 2.44 (s, 3H, H6), 2.26 (s, 3H, CH4), 2.19–2.02 (m, 2H, H^{Mt2}), 2.07 (s, 3H, SCH_3); ^{13}C NMR {^{1}H}: 175.3 (C4), 173.6 (C^{Mt1}), 160.3 (C6), 157.8 (C2), 108.2 (C5), 58.0 (C2'), 53.4 (C^{Mt2}), 47.5 (C1'), 29.2 (C^{Mt3}), 28.4 (C^{Mt4}), 23.0 (C4$\underline{C}H_3$), 19.57 (C6$\underline{C}H_3$), 13.46 (SCH_3). Found, %: C, 49.33; H, 7.37; N, 13.08; S, 9.98. $C_{13}H_{23}N_3O_4S$. Calculated, %: C, 49.19; H, 7.30; N, 13.24; S, 10.10.

A method for studying the hepatoprotective activity of substances on laboratory animals. To induce the toxic injury, after an 11-day course of the injections of a compound being tested, a 50% oily solution of a hepatotropic poison (CCl_4) was injected subcutaneously once a day for three days at a dose of 2 mL/kg 1–1.5 h after the injection of the compound. The antitoxic effect was assessed from the survival of animals, changes in the weight and body temperature during the injection of CCl_4 and after the last injection (Table 2). The hepatoprotective activity of the compounds was estimated as described in [7]. On the next day after the last combined injection of a compound and CCl_4, changes in the liver weight coefficient (the liver/body weight ratio), the morphometric parameters of histological sections, and the biochemical indices of blood serum, in particular, the level of ALT and AST, which are the markers of cytolysis, were examined. Animals were subjected to euthanasia under ether narcosis, the liver was withdrawn, and the weight coefficient of the liver was calculated and compared with the values in the control group. The antitoxic effect of the compounds was additionally estimated in the same animals by examining the weight of the right and left kidneys (weight coefficients of kidneys) (Table 3).

Body temperature was measured rectally using a Temperature Multiplexer device for the in vivo determination of temperatures in laboratory animals (TSE-Systems, Germany).

The biochemical analysis of blood serum was carried out on a Cobas Integra 400 analyzer (Rosch, Switzerland) using standard kits of reagents according to the manufacturers' recommendations. For the histo-

logical examination, paraffin slices were stained with hematoxylin and eosine. The morphometric analysis was performed under a Nikon microscope equipped with a Nikon digital camera using the NIS B software. In sections, the area of liver tissue affected by steatosis and necrosis was determined and expressed in percent relative to the total section area.

The statistical analysis of the results was performed using the programs Origin 6.1 and SPSS. In the case of a normal distribution, the data samplings were compared using the Student's *t*-test; and in the case of a nonnormal distribution, the Wilcoxon and Mann–Whitney nonparametric tests were used.

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