# THIOUREA AND THIOSEMICARBAZIDE DERIVATIVES: STRUCTURE, TRANSFORMATIONS, AND PHARMACOLOGICAL ACTIVITY. HEPATOPROTECTIVE EFFECT OF TRIAZINO- AND IMIDAZOINDOLES

**A. B. Tomchin,[1] S. V. Okovityi,[1] V. S. Velezheva,[1] and A. V. Smirnov[1]**

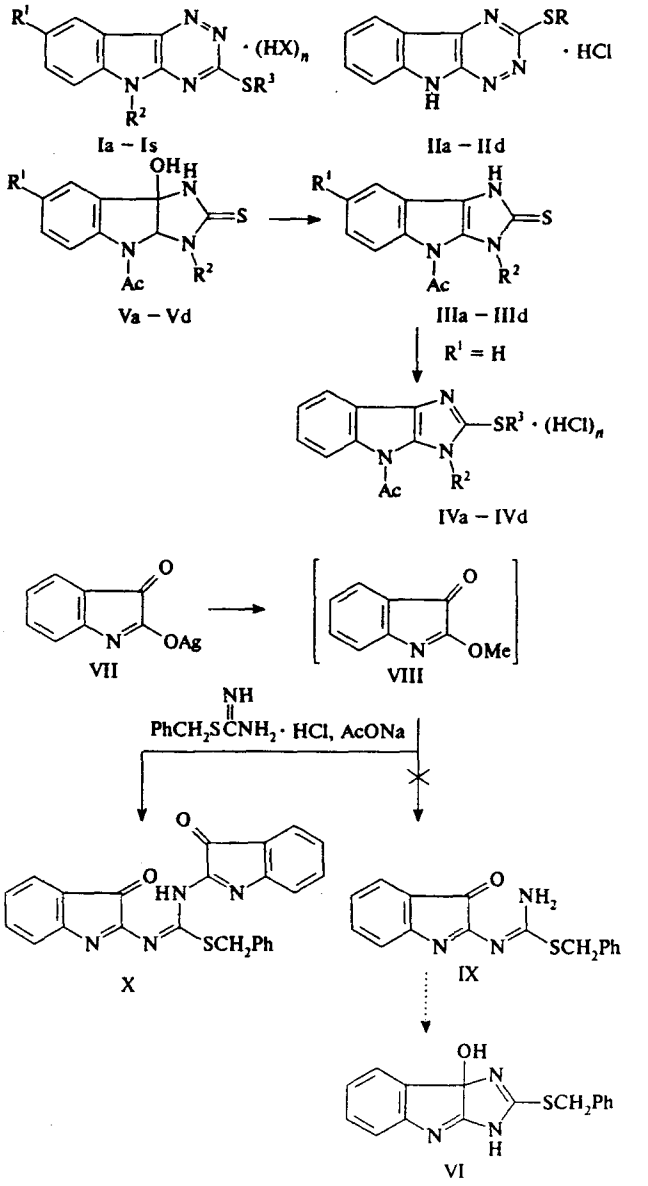
Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 32, No. 7, pp. 20 – 27, July, 1998.

*Original article submitted March 3, 1997.*

The efficacy of the well-known hepatoprotectors used for the treatment of liver disorders is insufficient [1]. Considering the permanent growth of traumatic, toxic, and infection-induced liver damage [2], it is currently of interest to search for new drugs possessing hepatoprotective activity. We have decided to carry out this search in the series of 1,2,4-triazinoindole derivatives with molecules containing thiourea fragments [3 – 5]. Since it is known that hepatoprotective activity is typical of antioxidants [6], these compounds are worth studying because of their ability to inhibit the process of lipid peroxidation and to produce stabilization of the membrane function [5]. Moreover, some compounds of this series are capable of suppressing the microsomal oxidation system of liver [7]. In this connection, we have grounds to expect the presence of hepatoprotective activity in these compounds, by analogy with the microsomal oxidation inhibitor dithiocarb and related preparations, which may be related to a decrease in the production of toxic metabolites of the hepatoprotective poisons [8]. In addition, we took into account that a possible way to accelerate the liver regeneration is to use the ability of triazinoindole derivatives to act upon the related immune mechanisms [9].

For this study, we have synthesized a series of 1,2,4-triazino[5,6-b]indole derivatives (Ia – Is) and isomeric 1,2,4-triazino[6,5]indole derivatives (IIa – Id). For the purpose of comparison, we have synthesized some other condensed indole derivatives also containing thiocarbamide fragments, including [4,5-b]indole-2-thiones (IIIa – IIId) and their S-alkyl derivatives (IVa – IVd). The syntheses of these compounds, except for Ik and On, were described elsewhere [3, 4, 10].

Compound In was obtained through the condensation of 1,3-bromochloropropane with 1-methylpiperazine, followed by interaction between the resulting 1-(4-methylpiperazino)-3-chloropropane with 2,3-dihydro-1,2,4-triazino[5,6-b]indole-3-thione in an alkaline medium. In order to compare the effect of various anions on the properties of salts, we isolated 3-(3-dimethylaminopropylthio)-1,2,4-triazino[5,6-b]indole in the form of hydrochloride (Ij) and malate (Ik).




[1] Military-Medical Academy, St. Petersburg, Russia.

Ia: $R^1 = R^2 = H$, $R^3 = (CH_2)_2NMe_2$, X = Cl, $n = 1$;
Ib: $R^1 = R^2 = H$, $R^3 = (CH_2)_2NEt_2$, X = Cl, $n = 1$;
Ic: $R^1 = R^2 = H$, $R^3 = (CH_2)_2NPr_2$, X = Cl, $n = 1$;
Id: $R^1 = R^2 = H$, $R^3$ = 2-(2-oxo-1-pyrrolidinyl)ethyl, $n = 0$;
Ie: $R^1$ = Br, $R^2$ = H, $R^3$ = 2-morpholinoethyl, X = Cl, $n = 2$, monohydrate;
If: $R^1$ = OMe, $R^2$ = H, $R^3$ = 2-morpholinoethyl, X = Cl, $n = 2$, monohydrate;
Ig: $R^1 = NH_2$, $R^2 = H$, $R^3 = (CH_2)_2NMe_2$, X = Cl, $n = 1$;
Ih: $R^1 = NH_2$, $R^2 = H$, $R^3 = (CH_2)_2NEt_2$, X = Cl, $n = 1$;
Ii: $R^1 = NH_2$, $R^2 = H$, $R^3$ = 2-morpholinoethyl, X = Cl, $n = 1$;
Ij: $R^1 = R^2 = H$, $R^3 = (CH_2)_3NMe_2$, X = Cl, $n = 1$;
Ik: $R^1 = R^2 = H$, $R^3 = (CH_2)_3NMe_2$, X = OOCCH(OH)$CH_2$COOH, $n = 1$;
Il: $R^1 = R^2 = H$, $R^3$ = 3-piperidinopropyl, X = Cl, $n = 1$;
Im: $R^1 = R^2 = H$, $R^3$ = 3-morpholinopropyl, X = Cl, $n = 1$;
In: $R^1 = R^2 = H$, $R^3$ = 3-3-(4-methylpiperazino)propyl, X = Cl, $n = 1$;
Io: $R^1 = R^2 = H$, $R^3$ = piperidinocarbonylmethyl, $n = 0$;
Ip: $R^1 = R^2 = H$, $R^3 = CH_2CON(Bu\text{-}i)_2$, $n = 0$;
Ir: $R^1 = H$, $R^2$ = 2-morpholinoethyl, $R^3$ = SPr, X = Cl, $n = 1$;
Is: $R^1 = H$, $R^2 = CH_2CONH_2$, $R^3$ = 2-morpholinoethyl, X = Cl, $n = 2$;
IIa: $R = (CH_2)_2NMe_2$,
IIb: $R = (CH_2)_2NEt_2$,
IIc: $R = (CH_2)_2NPr_2$,
IId: R = 2-morpholinoethyl;
IIIa, Va: $R^1 = R^2 = H$;
IIIb, Vb: $R^1$ = Br, $R^2 = H$;
IIIc, Vc: $R^1 = H$, $R^2$ = Me;
IIId, Vd: $R^1 = H$, $R^2 = CH_2Ph$;
IVa: $R^2 = H$, $R^3$ = Me, $n = 0$;
IVb: $R^2 = CH_2Ph$, $R^3$ = Me, $n = 0$;
IVc: $R^2$ = Me, $R^3 = (CH_2)_2NEt_2$, $n = 1$;
IVd: $R^2 = CH_2Ph$, $R^3 = (CH_2)_2NEt_2$, $n = 1$.

2-Alkylthioimidazo[4,5-b]indoles IVa – IVd were synthesized previously using the corresponding hydroxy derivatives Va – Vd [10]. In this work, we have attempted to obtain a compound of similar structure (VI) by an alternative method, proceeding from a silver salt of isatin (VII). Upon converting this salt into an O-methyl ester (VIII), we have performed condensation of this ester with S-benzylisothiourea in order to obtain isothiocarbamide IX with a view to its following cyclization with the formation of imidazoindole VI. However, we failed to isolate compound IX and instead obtained a compound of different structure (X), with molecules containing two indole fragments.

The proposed structure of compound X was confirmed by its mass spectrum containing a peak of the molecular ion and the most intense peak corresponding to scission of a benzyl fragment. Moreover, the $^1H$ NMR spectrum of this compound contains singlets due to one proton of the NH group and two protons of methylene group along with the signals of 13 aryl protons, which excludes the formation of compound IX. For a comparison with the condensed indole derivatives (I – VI) containing thiocarbamide fragment in a heteroring connected to the indole nucleus, we also performed biological tests on an indole derivative (X) containing the same fragment in a side chain linked to the indole nucleus. The yields, melting temperatures, and main parameters of the electronic absorption spectra of the newly synthesized compounds are listed in Table 1.

## EXPERIMENTAL CHEMICAL PART

The homogeneity of all compounds was checked, besides other methods, by TLC [11]. The absorption spectra were measured as described elsewhere [11]. The data of elemental analyses agree with the results of analytical calculations according to the empirical formulas.

**3-(3-Dimethylaminopropylthio)-1,2,4-triazino[5,6-b] indole malate (Ik).** To a solution of 10.11 g (50 mmole) of 2,3-dihydro-1,2,4-triazino[5,6-b]indole-3-thione (XI) [12] in 110 ml of a 1 N aqueous sodium hydroxide was added with stirring a solution of 8.29 g (52 mmole) of 3-dimethylaminopropyl chloride hydrochloride in 30 ml water. The mixture was stirred for 3 h at a temperature of 50°C and allowed to stand overnight. The precipitate was filtered, washed with water (2 × 25 ml) and acetone (30 ml), and dried at 80°C to obtain 12.08 g of base Ik. Recrystallization of the product from isobutyl alcohol (37 ml/g) yields (67.2%) of base Ik in the form of pale-yellow crystals; m.p., 210°C; UV spectrum in ethanol ($\lambda_{max}$, nm): 203 (log ε = 4.28), 223 (4.22), 265 (4.45), 345 (4.06); mass spectrum (*m/z*): 287 ($I_{rel}$ = 3% of maximum peak intensity) $[M]^+$, 242 (22) $[M–Me_2NH]^+$, 183 (15) $[M–Me_2NH–HNCS]^+$, 182 (13) $[M–Me_2NH–HNCS–H]^+$, 169 (16) $[M–S(CH_2)_3NMe_2]^+$, 168 (38) $[M–S(CH_2)_3NMe_2–H]^+$, 85 (85), 84 (99); $C_{14}H_{17}N_5S$.

A mixture of 2.5 g (8.70 mmole) of base Ik, 1.29 g (9.62 mmole) of malic acid, and 15 ml of absolute ethanol was heated until complete dissolution of the components. After the mixture was cooled to 20°C, the precipitate was filtered, washed by a dry ether (2 × 30 ml) and dried at 80°C to obtain 2.89 g (78.8%) of malate Ik; $R_f$ 0.63 (eluted with etha-

**TABLE 1.** Yields and Physicochemical Characteristics of the Synthesized Compounds

| Compound | Yield, % | M.p., °C (solvent for crystallization; amount, ml/g) | Empirical formula | UV $\lambda_{max}^{EtOH}$, nm (log ε) |
|---|---|---|---|---|
| Ik | 53[a] | 163 | $C_{14}H_{17}N_5S \cdot C_4H_6O_5$ | 203 (4.23), 223 (4.18), 265 (4.31), 345 (3.99) |
| In | 50.7[a] | 228 – 230 (ethanol – water, 2 : 1; 16.4) | $C_{17}H_{22}N_6S \cdot HCl$ | 224 (4.28), 266 (4.55), 348 (4.04) |
| IIc | 57.1[a] | 240[b] benzene – hexane, 1 : 1; 7.74); 7.74)[c] | $C_{17}H_{33}N_5S \cdot HCl$ | 268 (4.51), 297 sh (3.85), 337 (3.91), 409.5 (3.21) |
| X | 40.9[d] | 222 (butanol; 40) | $C_{24}H_{16}N_4O_2S$ | 258 (4.22), 285 sh (3.93), 3.99 (3.10) |

Notes: [a]Yield calculated relative to the corresponding 1,2,4-triazino-3-thione; [b]Melting temperature markedly depends on the heating rate. The sample was placed into the instrument at 230°C and heated at 3 K/min; [c]Solvent for recrystallization of the corresponding base; [d]Calculated relative to compound VII.

nol – concentrated aqueous ammonia mixture, 10 : 1). The proposed structure of Ik was confirmed by the results of potentiometric titration with an alkali in 70% ethanol and by the electronic absorption spectrum showing the peaks characteristic of the 3-alkylthio derivatives of 1,2,4-triazino[5,6-b]indole [3].

**3-[3-(4-Methylpiperazin-1-yl)propylthio]-1,2,4-triazino[5,6-b]indole hydrochloride (In).** To a solution of 90 g (0.9 mole) of 1-methylpiperazine and 91.08 g (0.9 mole) of triethylamine in 270 ml of anhydrous benzene was gradually added (over 3 h) with stirring a solution of 141.7 g (0.9 mole) of 1-bromo-3-chloropropane in 180 ml of anhydrous benzene, and the mixture allowed to stand overnight. The precipitate of triethylamine hydrochloride was filtered and washed with anhydrous benzene. The filtrate and washing liquid were combined and distilled to remove benzene. The residue was distilled in vacuum to yield 52.8 g (33%) of 1-(4-methylpiperazino)-3-chloropropane (XII) in the form of colorless transparent liquid (b.p., 85 – 86°C/7 Torr), which rapidly became turbid on storage. The isolated base is immediately involved in the synthesis and converted into a more stable hydrochloride. The hydrochloride (having the form of colorless hygroscopic crystals) is obtained at a nearly quantitative yield upon interaction of the base with a solution of anhydrous hydrogen chloride in ethyl ether.

To a solution of 19.42 g (95.6 mmole) of compound XI in 102 ml of 1 N aqueous sodium hydroxide was added 17.76 g (100.6 mmole) of base XII in 100 ml ethanol. The mixture was stirred for 8.5 h and allowed to stand overnight. Then the reaction mass was evaporated in vacuum and the residue was dried over calcium chloride in a desiccator, dissolved in 400 ml of boiling ethanol, cooled, and kept for 2 days. The precipitate was separated by filtration, suspended in a boiling mixture of 200 ml ethanol and 8 ml of concentrated HCl, and filtered hot. The product was mixed with 30 ml of ethyl ether, filtered, and dried in vacuum to obtain 15.73 g (43.5%) of raw compound In. The ethanol mother liquors standing for several days yield additionally in the form of precipitate 3.35 and 5.65 g of base In; m.p., 206°C (from ethanol). The total yield of compound In upon recrystallization amounts to 50.7%; $R_f$, 0.80 (eluted with ethanol – 3% aqueous ammonia mixture, 1 : 5). The product forms jelly structures with solvents, which makes it difficult to filter; compound In is readily soluble in water when cold, but is poorly soluble even upon boiling in ethanol, propanol, and glacial acetic acid. The proposed structure of In was confirmed by the same methods as Ik. The $^1$H NMR spectrum of In in DMSO-$d_6$ (δ, ppm): 2.20 (m, 2H, $CH_2$), 2.80 (s, 3H, $CH_3$), 3.30 – 3.60 (m, 12H, $6CH_2$), 7.40 (t, 1H, Ar), 7.60 – 7.80 (m, 2H, Ar), 8.30 (d, 1H, Ar), 11.80 (s, 1H, NH), 12.75 (s, 1H, $NH^+$); the $^1$H NMR spectrum in $D_2O$ (δ, ppm): 2.22 (m, 2H, $CH_2$), 3.00 (s, 3H, $CH_3$), 3.14 (m, 2H, $CH_2$), 3.42 (m, 4H, $2CH_2$), 3.70 (m, 6H, $3CH_2$), 6.85 – 6.97 (m, 2H, Ar), 7.22 – 7.42 (m, 2H, Ar).

**3-(2-Dipropylaminoethylthio)-1,2,4-triazino[6,5-b]indole hydrochloride (IIc).** To a solution of 5 g (24.75 mmole) of 2,3-dihydro-1,2,4-triazino[6,5-b]indole-3-thione [13] was added over 30 min with intensive stirring a solution of 5.83 g (32.14 mmole) of 2-dipropylaminoethyl chloride hydrochloride in 15 ml of water and the mixture was allowed to stand overnight. The precipitate was filtered, washed with water (2 × 10 ml) and dried in vacuum to obtain 6.65 g of base IIc; yield upon recrystallization, 5.42 g (66.5%). To a mixture of the product with 30 ml of ethanol was added 1.57 ml of concentrated HCl and the mixture was boiled for 2 min and cooled to 20°C. Then 80 ml of ethyl ether was added and the mixture was kept for 2 h at – 10°C. The precipitate was filtered, washed with ether (6 × 5 ml), and dried to yield 4.2 g of compound IIc in the form of lemon-yellow crystals soluble in water.

**N,N'-Di(3-oxo-2-indolinyl)-S-benzylthiourea (X).** A mixture of 32 g (126 mmole) thoroughly dried and finely ground compound VII with 70 ml anhydrous ethyl ether and 12.2 ml (101.2 mmole) of methyl iodide was kept for 9 days (shaken a few times) without access of light and moisture. Then 85 ml of anhydrous benzene was added and the mixture was vigorously shaken for 3 min and kept for 1 h in the dark. The precipitate was filtered and washed with anhydrous benzene (3 × 30 ml). The washing liquid was combined with the filtrate. A rapidly prepared solution of 23 g (113 mmole) S-benzylisothiourea and 15.4 g (113 mmole) sodium acetate trihydrate in 740 ml of water heated to 50°C was mixed with the above ether – benzene solution. The mixture was vigorously shaken for 3 h and allowed to stand overnight. The precipitate was filtered, and washed sequentially with benzene (2 × 8 ml), water (4 × 20 ml), and pentane (5 × 20 ml), and dried in vacuum over phosphoric anhydride and paraffin. This yields bright reddish crystals of compound X; $R_f$, 0.44 (eluted with ethyl acetate – hexane mixture, 1 : 2); $^1$H NMR spectrum in DMSO-$d_6$ (δ, ppm): 4.11 (s, 2H, $CH_2$), 6.41 (d, 1H, J 9 Hz, Ar), 6.98 – 7.81 (m, 12H, Ar), 8.27 (s, 1H, NH); mass spectrum (*m/z*): 424 (16.4) ($I_{rel}$, %) $[M]^+$, 333 (100) $[M-CH_2C_6H_5]^+$, 305 (12.7) $[M-CH_2C_6H_5-CO]^+$, 301 (7.5) $[M-CH_2C_6H_5-S]^+$, 278 (10), 277 (7.8), 248 (10.7), 220 (5.8), 189 (8.4), 147 ((9.1), 146 (9.1), 145 (52.7).

T. I. Zhukova and T. A. Kuznetsova participated in the syntheses of compounds Ik and In.

## EXPERIMENTAL BIOLOGICAL PART

The synthesized compounds were tested on a group of white male mongrel rats weighing 130 – 200 g. The toxic damage to the rat liver was induced by subcutaneous injections of a 50% tetrachloromethane solution in vaseline oil at a dose of 0.4 ml/100 g body weight; the injections were made every day during a four-day period [14]. Animals in the test groups were simultaneously (during the same four-day period) intraperitoneally injected with the synthesized compounds either dissolved in a physiological solution or sus-

pended with Tween-80 additives (1 ml per animal). Animals in the control group were injected with the physiological solution. Each group contained 6 – 10 animals. Doses producing the maximum hepatoprotective effect were determined in the series of preliminary experiments. The reference drug preparations were represented by a combination of riboxine and potassium orotate (each 50 mg/kg) [15] and essentiale (80 mg/kg) [16]. The materials for biochemical analyses were taken on the seventh day after the onset of tetrachloromethane intoxication, since it is known that the most pronounced morphological and functional changes are typically developed by this time [17].

We have also studied the effects of some compounds on the process of liver regeneration using the model of partial hepatectomy, which provides maximum stimulation of the hepatocyte proliferation [18]. For this purpose, the left side and central lobes of the liver (2/3 of the whole organ) were removed from animals under ether narcosis. The operated animals were divided into test and control groups, each including 6 – 10 rats. The synthesized compounds and reference drugs were injected intraperitoneally on the day of operation and during the following four days (animals in the the control group were injected with physiological solution), since it is known that a maximum intensity of the process of protein and nucleic acid synthesis is observed within a four-day period after hepatectomy [19]. The rats were killed on the seventh day, by which time the liver regeneration process virtually terminated [20].

The effect of compound In was also studied on the model of experimental chronic hapatitis [21] in a group of male rats weighing 180 – 200 g. The hepatitis was induced by the peroral introduction of tetrachloromethane (0.2 ml/kg) in a 50% oil solution three times per week during a 30-day period. Animals in the test group were every day intraperitoneally injected with compound In at a dose of 50 mg/kg. The initial solution of compound In (10 mg/ml) was preliminarily adjusted at pH 3.5 with 0.1 N sodium hydroxide solution with Tween-80 additives. The test and control groups contained six animals each. The diagnostics of liver diseases is most frequently based on determination of the activity of alanine aminotransferase (AlAT) and aspartate aminotransferase (AsAT), because the necrosis of hepatocytes results in the release of these enzymes into the blood channel, leading to an increase in their concentration in the blood serum [17]. The contents of AlAT and AsAT in the blood serum and the total bilirubin, total protein, urea nitrogen, creatinine, glucose, and potassium were determined using the automated biochemical analyzers SMA-12/16 (Technicon Instruments, USA) and Abbot-Spectrum (Abbot Laboratories s.a., Switzerland).

On the seventh day after the onset of tetrachloromethane intoxication or upon the partial hepatectomy, or on the 31st day for the chronic tetrachloromethane administration, the degree of recovery of the functional activity of liver was characterized by determining the hexenal-induced sleep duration. Hexenal was introduced intraperitoneally at a dose of 80 mg/kg [22]. After termination of the hexenal sleep, the animals were killed by decapitation and the samples of liver and blood were taken for the biochemical analysis.

The results of experiments were statistically processed by the methods of variational statistics on an IBM PC/AT personal computer using the STATGRAPHICS program package. The data were also treated by the nonparametric methods using the Wilcoxon – Mann – Whitney criterion.

The hepatoprotector activity was characterized by ratios of the values of parameters in the control group to the corresponding values in the test group. In particular, the effects of the synthesized compounds on the AlAT, AsAT, and bilirubin concentrations and the hexenal sleep duration were described by the coefficients $K_{AlAT}$, $K_{AsAT}$, $K_{bil}$, and $K_{hex}$. In order to reveal a possible mutual dependence of these quantities, we used the model of tetrachloromethane hepatitis (where a broad circle of compounds was tested) to calculate an approximate model relationship valid for all compounds except If, Ir, and IIc:

$$K_{AlAT} = 2.03\,K_{AsAT} - 1.36\,K_{AsAT}^2 + 0.322\,K_{AsAT}^3, \quad (1)$$

with $F = 454.2$, $p \le 0.0001$, $r = 0.98$ (all coefficients are statistically significant for $p \le 0.0001$). Thus, the values of $K_{AlAT}$ and $K_{AsAT}$ are reliably interrelated. As is seen from the data presented in Tables 2 and 3, the $K_{AlAT}$ value is more sensitive to changes in the chemical structure than is $K_{AsAT}$. For this reason, we have used $K_{AlAT}$ as a quantity describing the drug effect on the transaminase level in an integral assessment of the hepatoprotective activity. For the assessment, the synthesized compounds were characterized by an integral parameter, called the protection coefficient (PC), calculated as the arithmetic mean of three characteristic quantities: $PC = (K_{AlAT} + K_{bil} + K_{hex})/3$.

## RESULTS AND DISCUSSION

As is seen from Table 2, the tetrachloromethane intoxication leads to cytopathologic and general functional changes in the rat liver. The AlAT concentration in the control group and the total bilirubin in the blood plasma increase by a factor of 2.3 and 2, respectively, as compared to their levels in the intact group. Also markedly affected is the function of the microsomal oxidation system: the hexenal-induced sleep duration increases 2.9 times as a result of decrease in the detoxicating function of liver.

All series of the compounds studied, including indole, 1,2,4-triazino[5,6-b]indole, 1,2,4-triazino[6,5]indole, and imidazo[4,5-b]indole derivatives, contain compounds possessing high hepatoprotective activity.

For the model of acute tetrachloromethane-induced hepatitis, the AlAT level in the blood plasma was found to decrease (against the control) under the action of compounds Id ($K_{AlAT} = 1.3$), Ig (2.03), Ih (1.66), Ii (1.46), Im (1.32), In (2.0), Ir (2.89), IIc (2.02), IIIa (2.16), Va (1.18), and X (2.3).

The AsAT concentration decreased under the action of compounds Id ($K_{AsAT}$ = 1.26), Ig (1.28), Im (1.70), In (1.29), IIIa (1.38), and X (1.27). Compounds Ih, IIc, and Va have virtually no effect on this parameter, while compound Ir even increases the AsAT content in the blood plasma by a factor of 1.28 against the control.

The total bilirubin level in the blood plasma was decreased by compounds Im ($K_{bil}$ = 1.86), IIc (1.53), and Va (2.0), while compounds Id, Ig, Ih, Ii, In, and X produced no effect on this parameter, and compounds Ir and IIIa increased the level of bilirubin by a factor of 2.5 and 2, respectively.

The hexenal-induced sleep duration was reduced by compounds Id ($K_{hex}$ = 1.9), Ig (1.64), Ih (1.28), Ii (1.7), Im (1.98), In (1.52), IIIa (1.61), and X (1.82). Compounds Ir and IIc did not affect this parameter, while compound IVa actually increased the hexenal sleep duration by a factor of 1.23.

Study of the effect of nine compounds on animals upon partial hepatectomy revealed two compounds (In and Is) possessing sufficiently pronounced hepatoprotective activity (Table 3). These drugs decrease the AlAT content in the blood plasma by a factor of 1.23 and 1.7, and the total bilirubin, by a factor of 1.66 and 1.2, respectively. The AsAT level and the hexenal sleep duration were not significantly affected by these compounds.

For the model of experimental chronic hepatitis in rats, compound In (one of the most active) reliably reduced the level of AlAT [from 286.3 ± 6.8 to 195.0 ± 9.9 mmole/(sec • liter)], bilirubin (from 5.13 ± 0.2 to 3.42 ± 0.11 μmole/liter),

**TABLE 2.** Effect of Indole Derivatives on the Functional-Metabolic Characteristics of Liver Upon Acute Intoxication with Tetrachloromethane

| Compound | Dose, mg/kg (i.p.) | AlAT in plasma, mmole/(sec · liter) | AsAT in plasma, mmole/(sec · liter) | Total bilirubin, μmole/liter | Hexenal sleep, min | $PC_1$ |
|---|---|---|---|---|---|---|
| Intact animals | – | 59.7 ± 3.35 | 30.2 ± 5.20 | 3.40 ± 0.27 | 17.5 ± 1.9 | – |
| Control | – | 137.8 ± 8.76** | 83.2 ± 6.25** | 6.84 ± 0.13** | 50.0 ± 5.2** | – |
| Riboxine + potassium orotate | 100 | 99.2 ± 6.30* | 60.1 ± 7.15* | 6.32 ± 0.31 | 38.5 ± 3.7* | 1.28 |
| Essentiale | 80 | 92.5 ± 10.2 | 75.0 ± 6.30 | 5.16 ± 0.20 | 33.6 ± 2.67 | 1.43 |
| Ia | 42 | 263.3 ± 18.92* | 108.1 ± 11.01 | 4.52 ± 0.26* | 46.3 ± 4.1 | 1.04 |
| Ib | 46 | 286.8 ± 17.08* | 105.6 ± 11.23 | 4.56 ± 0.18* | 53.5 ± 4.7 | 0.98 |
| Ic | 50 | 95.7 ± 9.93 | 96.7 ± 13.15 | 12.91 ± 2.7 | 43.9 ± 1.3 | 1.05 |
| Id | 50 | 106.0 ± 6.74* | 66.0 ± 6.16 | 6.80 ± 0.40 | 26.3 ± 2.8* | 1.40 |
| Ie | 20 | 82.5 ± 10.2 | 79.2 ± 7.41 | 6.84 ± 0.37 | 55.0 ± 5.0 | 1.19 |
| If | 59 | 104.4 ± 6.64* | 57.8 ± 3.69* | 6.88 ± 0.42 | 42.7 ± 5.5 | 1.16 |
| Ig | 50 | 67.9 ± 4.30* | 65.0 ± 6.05 | 6.64 ± 0.29 | 30.5 ± 2.6* | 1.57 |
| Ih | 50 | 83.0 ± 5.28* | 88.2 ± 4.91 | 6.84 ± 0.38 | 39.0 ± 3.4* | 1.31 |
| Ii | 50 | 94.4 ± 6.00* | 85.7 ± 8.54 | 6.82 ± 0.38 | 29.4 ± 3.2* | 1.39 |
| Ij | 50 | 101.3 ± 16.7 | 108.0 ± 14.1 | 10.70 ± 0.98 | 37.6 ± 4.1 | 1.11 |
| Ik | 50 | 128.8 ± 12.1 | 99.8 ± 7.64 | 4.52 ± 0.18* | 52.5 ± 3.8 | 1.18 |
| Il | 6 | 398.3 ± 26.1* | 158.0 ± 7.51* | 6.80 ± 0.36 | 64.0 ± 5.8 | 0.77 |
| Im | 40 | 104.4 ± 4.02 | 48.9 ± 4.5 | 3.66 ± 0.15 | 26.6 ± 2.4 | 1.69 |
| In | 50 | 68.9 ± 4.63 | 64.5 ± 3.21 | 6.85 ± 0.34 | 32.9 ± 2.8* | 1.51 |
| Io | 50 | 125.3 ± 8.62 | 81.6 ± 6.99 | 8.55 ± 1.75 | 36.8 ± 6.8 | 1.09 |
| Ip | 50 | 139.2 ± 8.94 | 84.8 ± 3.07 | 6.79 ± 0.27 | 53.0 ± 5.4 | 0.98 |
| Iq | 50 | 47.69 ± 6.03* | 106.1 ± 9.2 | 17.1 ± 2.12* | 50.0 ± 3.6 | 1.43 |
| IIa | 42 | 269.1 ± 17.5* | 121.8 ± 10.15 | 4.56 ± 0.22* | 51.5 ± 3.3 | 0.99 |
| IIb | 46 | 271.5 ± 14.4* | 120.6 ± 10.16* | 4.58 ± 0.22* | 51.0 ± 4.9 | 0.99 |
| IIc | 50 | 68.2 ± 4.35 | 84.0 ± 5.45 | 4.48 ± 0.16* | 42.4 ± 5.4 | 1.58 |
| IId | 48 | 264.6 ± 16.81* | 122.3 ± 17.36* | 4.60 ± 0.17* | 41.6 ± 3.6 | 1.07 |
| IIIa | 50 | 63.8 ± 6.06 | 60.3 ± 8.16* | 13.7 ± 0.98* | 31.0 ± 3.0* | 1.42 |
| IIIb | 20 | 123.1 ± 7.79 | 78.5 ± 8.11 | 4.66 ± 0.18* | 70.0 ± 7.2 | 1.10 |
| IVb | 25 | 165.4 ± 10.51* | 67.6 ± 9.21 | 3.24 ± 0.07* | 39.8 ± 4.1 | 1.40 |
| IVc | 25 | 140.6 ± 8.93 | 76.3 ± 5.23 | 6.80 ± 0.27 | 42.4 ± 4.4 | 1.05 |
| IVd | 25 | 147.5 ± 12.22 | 77.7 ± 8.04 | 4.36 ± 0.11* | 71.4 ± 6.8* | 1.07 |
| Va | 50 | 115.8 ± 10.36 | 94.0 ± 4.63 | 3.42 ± 0.16* | 61.5 ± 5.2 | 1.33 |
| X | 50 | 59.9 ± 3.35 | 65.5 ± 9.36 | 6.78 ± 0.34 | 27.5 ± 4.3* | 1.37 |

Notes (here and in Table 3): * difference from control statistically reliable for $p \leq 0.05$; ** difference from intact group statistically reliable for $p \leq 0.05$.

and alkaline phosphatase (from 444.4 ± 20.6 to 183.7 ± 14.3 μmole/ml), and produced a 20% increase in the survival of test animals. For this model, the protection coefficient amounts to 1.3. Thus, the hepatoprotective activity of compound In is also confirmed on the model of experimental chronic hepatitis.

An analysis of data for the model of acute tetrachloromethane-induced liver damage reveals a correlation between the effect of the triazinoindole derivatives (except for compounds Id, Ii, Im, and Ir) on the AtAT activity and the hexenal sleep duration:

$$K_{hex} = 3.33K_{AlAT} - 2.99K^2_{AlAT} + 0.84K^3_{AlAT}, \quad (2)$$

with $F = 247.5$, $p \le 0.0001$, $r = 0.978$ (all coefficients are statistically significant for $p \le 0.0001$). However, this relationship is valid for a smaller number of compounds and is less strict compared to the above relationship between $K_{AlAT}$ and $K_{AsAT}$. According to equation (2), an increase in $K_{AlAT}$ in the interval studied may both increase and decrease the value of $K_{hex}$. Therefore, it would be expedient to consider the two as independent.

The values of protection coefficient determined on the two models (denoted by subscripts 1 and 2 according to the equation number) for the 1,2,4-triazino[5,6-b]indole derivatives exhibit a linear relationship: $PC_2 = 1.807\ PC_1^2 - 0.811\ PC_1^3$ ($F = 4625$). The corresponding correlations are also established for the effects of these compounds on the particular functional-metabolic characteristics of the liver (note that the two last relationships are linear): $K_{AlAT(2)} = 1.695K^2_{AlAT(1)} - 0.706K^3_{AlAT(1)}$ (except for compounds V; $F = 9545$); $K_{bil(2)} = 1.003\ K_{bil(1)}$ (except for compound Im; $F = 192{,}183$); $K_{hex(2)} = 0.986\ K_{hex(1)}$ (except for compound Ie; $F = 5565$). For the latter four correlations, $p \le 0.00001$, $r \ge 0.999$, and all coefficients are statistically significant with $p \le 0.0001$ in the relationship for $K_{AlAT}$ and with $p \le 0.00001$ in the relationships for other quantities.

An analysis of the experimental data revealed several relationships between the structure of compounds and their hepatoprotective activity. In the series of dialkylaminoethylthio derivatives of 1,2,4-triazino[5,6-b]- and [6,5-b]indole, the compounds having terminal dimethylamino and diethylamino groups are inactive, while the transition to dipropylamino group imparts certain activity to the compound. High activity is observed for the derivatives of 2-pyrrolidone, 1-methylpiperazine, and morpholine, that is, compounds with side chains containing the residues of cyclic amines. On the contrary, the presence of a piperidine residue in the side chain or the substitution of a carbonyl group for one of the two methylene units linked to a side-chain amino group lead to a decrease in the activity, while introduction of the third methylene unit increases the hepatoprotective effect.

The substitution of bromine or methoxy group for hydrogen in position 8 of the triazinoindole derivatives studied does not significantly influence the activity, while the introduction of amino group leads to compounds If–Ih distinguished by high activity. The latter compounds usually do not obey the structure–activity relationships considered below. Therefore, we suggest that the amino group in position 8 directly interacts with the corresponding receptor.

The hepatoprotective activity was retained or increased upon substitution of aminocarbonylmethyl or morpholinoethyl groups for hydrogen at the indole nitrogen. A comparison of the data for 1,2,4-triazino[5,6-b]indole and 1,2,4-triazino[6,5-b]indole derivatives shows that the activity is not strongly affected by the condensation site of indole and triazine nuclei, except for the dipropylaminoethyl derivatives (where compounds of the latter series are more active).

As is seen from the data for compounds Ij and Ik, the activity of salts depends on the structure of both cation and anion. The malate (Ik) is somewhat more active than the hydrochloride (Ij). However, the latter compound increases the level of creatinine in the blood plasma (1.5 times against the control level), which is evidence of a disruption of the excretion function of kidneys.

In the series of imidazo[4,5-b]indole derivatives, a significant protective effect is observed for both unsubstituted 2-thiones and their S-methyl substituted analogs, the latter exceeding in activity the S-diethylaminoethyl analogs. With

**TABLE 3.** Effect of Indole Derivatives on the Functional-Metabolic Characteristics of Liver Upon Partial Hepatectomy

| Compound | Dose, mg/kg (i.p.) | AlAT in plasma, mmole × $sec^{-1} \cdot liter^{-1}$ | AsAT in plasma, mmole × $sec^{-1} \cdot liter^{-1}$ | Total bilirubin, μmole/liter | Hexenal sleep, min | $PC_1$ |
|---|---|---|---|---|---|---|
| Intact animals | – | 45.3 ± 1.70 | 54.0 ± 3.20 | 2.05 ± 0.09 | 22.3 ± 1.0 | – |
| Control | – | 96.5 ± 28.0** | 74.9 ± 12.2** | 2.74 ± 0.02** | 62.0 ± 8.0** | – |
| Riboxine + potassium orotate | 100 | 65.0 ± 1.0* | 56.0 ± 0.9* | 2.58 ± 0.12 | 50.2 ± 6.5 | 1.26 |
| Ic | 50 | 66.8 ± 14.52 | 86.9 ± 1.92 | 5.13 ± 0.17* | 59.0 ± 3.43 | 1.01 |
| Ie | 20 | 67.5 ± 9.58 | 79.4 ± 1.22 | 2.70 ± 0.08 | 50.4 ± 2.59 | 1.22 |
| Ij | 50 | 70.7 ± 2.58 | 97.9 ± 2.53* | 4.28 ± 0.85* | 46.5 ± 10.5 | 1.11 |
| Im | 40 | 73.2 ± 7.67 | 69.3 ± 1.67 | 5.13 ± 0.68* | 33.0 ± 2.01* | 1.24 |
| In | 50 | 78.4 ± 2.17 | 64.6 ± 6.5 | 1.65 ± 0.03* | 63.5 ± 1.89* | 1.29 |
| Io | 50 | 87.6 ± 2.90 | 73.4 ± 2.53 | 3.42 ± 0.86 | 45.5 ± 7.49 | 1.09 |
| Is | 12.5 | 56.7 ± 6.67* | 71.3 ± 6.43 | 2.28 ± 0.16* | 56.4 ± 6.02 | 1.33 |
| IIIa | 50 | 88.5 ± 4.22 | 66.9 ± 5.76 | 3.34 ± 0.04* | 75.6 ± 8.36 | 0.91 |
| IVa | 50 | 70.4 ± 2.44 | 72.7 ± 11.88 | 2.70 ± 0.21 | 44.3 ± 5.56* | 1.26 |

substitution of bromine for hydrogen in position 7, the activity decreases. At the same time, with the substitution of benzyl radical for hydrogen in the imidazole ring, the activity is retained. Dehydration of the hydroxy derivatives of imidazoindole (Va – Vd) leads to an increase in the activity. The isothioureide indole derivative X (containing no additional condensed heterorings) also exhibits activity.

We have investigated the relationship between the hepatoprotective activity of the synthesized compounds and their most important physicochemical properties including the ionization constants and the distribution coefficients in the octanol – water system [3, 4].

The group of water-soluble 1,2,4-triazino[5,6-b]indoles (except for the 8-amino derivatives) is characterized by a dome-shaped plot of basicity versus activity, which is well described by the third-power parabola equation: $PC_I = 0.0853\ pK_a^2 - 0.00833\ pK_a^3$ ($F = 193.8$; $p \le 0.0001$; $r = 0.98$). All coefficients are statistically significant for $p \le 0.0001$. Therefore, there exists an optimum basicity for these compounds that corresponds to the interval $pK_a$ 6.85 ± 1. An analysis of the data for all compounds without exclusions shows that the maximum activity is usually observed in the region of low basicity ($pK_a < 7$). This circumstance hinders the selection of compounds with sufficiently high solubilities appropriate for the preparation of injection medicinal forms. In this context, the 8-amino derivatives are worthy of special attention, since their optimum basicity interval is shifted toward higher $pK_a$ values; another promising group includes compounds Im, In, and IIc.

No correlation was observed between the integral hepatoprotective activity parameters of the synthesized compound and their lipophilicity. The active compounds show a broad range of lipophilicities, whereby the $\log P_{ap}$ values vary from 2.1 to 4.3. Here, an exception is again presented by the 8-amino derivatives whose optimum lipophilicity interval is shifted toward lower $\log P_{ap}$ values.

Besides considering a combination of activity parameters as described by the protection coefficient (PC), it is also of interest to analyze relationships between the structure and particular functional-metabolic characteristics of the liver. In the model of acute tetrachloromethane intoxication, the cytolytic syndrome characteristics are most noticeably decreased by compounds Ie, Ig, Ih, In, Ir, IIc, IIIa, and X, while in the model of hepatectomy, the maximum effect is produced by compound Is (all these compounds decrease the AlAT concentration by a factor of 1.6 – 2.9). The series of 1,2,4-triazino[5,6-b]indoles (except for compounds Ij, Il, Ir, and the 8-amino derivatives) exhibits a relationship between lipophilicity and their effect on the AlAT level: $K_{AlAT(l)} = 0.517 \log P_{ap}$ ($F = 143.3$; $p \le 0.0001$; $r = 0.95$). All coefficients are statistically significant for $p \le 0.00001$.

According to the first model, the function of bilirubin formation is most significantly improved by compounds Ia, Ib, Ik, Im, IIa – IId, IVb, IVd, and Va. In the second model, the maximum effect is observed for compound In reducing the bilirubin concentration in the blood serum 1.5 – 2.1 times against the control level. In this respect, the activity is inherent in the 2-dimethylamino and 2-diethylaminoethylthio derivatives of both triazinoindole isomer series. The 1,2,4-triazino[6,5-b]indole derivatives IIc and IId exceed in activity the corresponding compounds of the isomer series. Similarly to the PC values, the compounds belonging to both isomer series (except for the 8-amino derivatives and compounds Id, Il, and IIc) are characterized by a dome-shaped plot of $K_{bil}$ versus lipophilicity: $K_{bil(l)} = 1.08 \log P_{ap} - 0.0919 \log P_{ap}^3$ ($F = 101.7$; $p \le 0.0001$; $r = 0.94$). All coefficients are statistically significant for $p \le 0.00001$. The values of optimum lipophilicity for the bilirubin formation range within $\log P_{ap} = 1.9 \pm 0.4$.

It should be noted that the above correlations are more reliably established for the apparent distribution coefficients $\log P_{ap}$ than for the $\log P$ values [3]. This fact is indicative of a considerable contribution from ionization. Indeed, the soluble compounds of the 1,2,4-triazino[5,6-b]indole series (except for the 8-amino derivatives and compound Ic) show a reliable correlation between the bilirubin level and ionization constants: $K_{bil(l)} = 0.0133\ pK_a^3 - 0.00132\ pK_a^4$ ($F = 54.6$; $p \le 0.0001$; $r = 0.93$). All coefficients are statistically significant for $p \le 0.0016$. The optimum $pK_a$ value for the bilirubin formation in the presence of soluble compounds is 7.5 ± 0.3.

In the model of tetrachloromethane hepatitis, the activity of the microsomal oxidation system of liver is most significantly improved by compounds Id, Ig, Ii, Im, In, IIIa, and X, while in the partial hepatectomy model, the maximum effect is observed for compound Im (all these compounds increase the hexenal sleep duration by a factor of 1.5 – 1.9). The soluble compounds of the 1,2,4-triazino[5,6-b]indole series (except for the 8-amino derivatives) show a reliable correlation between this parameter and the basicity parameter: $K_{hex(l)} = 0.351\ pK_a - 0.0262\ pK_a^2$ ($F = 156.9$; $p \le 0.0001$; $r = 0.96$). All coefficients are statistically significant for $p \le 0.0035$. The optimum $pK_a$ value for the parameter under consideration is 6.7 ± 0.3.

It was interesting to compare the hepatoprotective activity of the synthesized compounds to the other types of their activity studied earlier. No correlation is observed between the hepatoprotective properties and antihypoxic activity inherent in many compounds in the series studied [3, 4, 23, 24]. It was noted above that some compounds are also capable of inhibiting the microsomal oxidation in the early stage upon administration [7]. As expected, these compounds exhibited high hepatoprotective activity, which is probably related to a decrease in the formation of toxic metabolites of tetrachloromethane. In our experiments, the duration of the hexenal-induced sleep was observed to decrease on the seventh day after beginning the administration of tetrachloromethane and the compounds studied, which is evidence for restored ability of the liver to metabolize xenobiotics. There is a qualitative relationship between hepatoprotective activity and the actoprotective effect. In particular, a high protective activity with

respect to the liver function is observed for the triazinoindole and imidazoindole derivatives Ie, Im, Ii, Is, and IVa, which are also capable of increasing or restoring the capacity to work after the action of exhaustive loads or extremal factors [25, 26]. In the model of partial hepatectomy, the maximum activity is also typically observed for the compounds possessing actoprotective properties.

On the basis of the results obtained in this work, we may conclude on the good prospects of searching for new hepatoprotectors in the series of indole, 1,2,4-triazino[5,6-b]indole, 1,2,4-triazino[6,5]indole, and imidazo[4,5-b]indole derivatives.

## REFERENCES


1. Yu. N. Chernov and M. V. Vasin, in: *Pharmacological Correction of Hypoxic States. Proc. of the 2nd All-Union Conf.* [in Russian], Grodno (1991), p. 495.
2. S. D. Podymova, *Liver Diseases. A Guide for Therapists* [in Russian], Meditsina, Moscow (1993).
3. A. B. Tomchin, O. Yu. Uryupov, T. I. Zhukova, et al., *Khim.-Farm. Zh.*, **31**(3), 19 – 27 (1997).
4. A. B. Tomchin, O. Yu. Uryupov, and A. V. Smirnov, *Khim.-Farm. Zh.*, **31**(12), 6 – 11 (1997).
5. A. B. Tomchin and A. V. Kropotov, *Khim.-Farm. Zh.*, **32**(1), 22 – 26 (1998).
6. N. P. Skakun and I. Yu. Vysotskii, *Antibiotiki*, No. 3, 223 – 227 (1984).
7. N. N. Pluzhnikov, O. Yu. Uryupov, A. B. Tomchin, et al., *Vestn. Len. Gos. Univ., Ser. 3*, **2**(10), 50 – 55 (1993).
8. A. I. Vengerovskii and A. S. Saratikov, *Farmakol. Toksikol.*, No. 1, 89 – 93 (1988).
9. I. N. Yanvareva, O. Yu. Uryupov, V. N. Aleksandrov, and A. B. Tomchin, *Vestn. Len. Gos. Univ.*, Ser. 3, **2**(10), 59 – 64 (1990).
10. V. S. Velezheva, A. B. Tomchin, A. I. Mel'man, et al., *Zh. Org. Khim.*, **34**(4), 604 – 617 (1998).
11. A. B. Tomchin, *Zh. Org. Khim.*, **28**(10), 2207 – 2213 (1992).
12. I. S. Ioffe, A. B. Tomchin, and E. N. Zhukova, *Zh. Obshch. Khim.*, **39**(1), 78 – 83 (1969).
13. I. S. Ioffe, A. B. Tomchin, and G. A. Shirokii, *Zh. Org. Khim.*, 7(1), 179 – 181 (1971).
14. N. V. Lazarev (ed.), *Reproduction of Diseases in Animals for Experimental-Therapeutical Investigations* [in Russian], Medgiz, Leningrad (1954).
15. V. E. Rychnev and V. M. Frolov, *Use of Regeneration Stimulators in the Therapy of Viral Hepatitis and Other Liver Diseases* [in Russian], Voronezh State Univ., Voronezh (1984).
16. A. I. Vengerovskii, V. S. Chuchalin, and O. V. Paul's, *Byull. Eksp. Biol. Med.*, No. 8, 192 – 195 (1987).
17. T. F. Slater, *Biochemical Studies of Liver Injury*, Academic Press, London (1978), pp. 1 – 45.
18. G. M. Higgins and R. M. Anderson, *Arch. Pathol.*, **12**(1), 188 – 202 (1931).
19. B. P. Solopaev, *Regeneration of Normal and Pathologically Modified Liver* [in Russian], Volgo-Vyatsk. Khizhn. Izdat., Gorkii (1980).
20. V. F. Sidorova, Z. A. Ryabinina, and E. M. Leikina, *Regeneration of Liver in Mammals* [in Russian], Medgiz, Leningrad (1966).
21. A. S. Saratikov, A. I. Vengerovskii, N. O. Baturina, et al., *Eksp. Klin. Farmakol.*, **59**(1), 24 – 26 (1995).
22. W. C. Verly, *The Control of Liver Growth*, Chalones, New York (1976).
23. A. B. Tomchin, V. M. Vinogradov, T. I. Zhukova, et. al., USSR Inventor's Certificates Nos. 940476, 978567, 1014249 (1981); 1233460, 1231835, 1235181 (1984); 1487413, 1498021 (1987); 1547281, 1596719 (1988); 1598431, 1665679, 1681504 (1989); 1804067 (1991), *Byull. Izobret.*, No. 25 (1996).
24. A. B. Tomchin, V. M. Vinogradov, and T. S. Basieva, USSR Inventor's Certificates Nos. 1014250, 1032758 (1981); 1139143 (1983), *Byull. Izobret.*, No. 26 (1996).
25. A. B. Tomchin, V. M. Vinogradov, R. P. Spivakova, et. al., USSR Inventor's Certificates Nos. 1220301 (1984); 1498021 (1987); 1584352, 1584382 (1988); 1809608 (1991), *Byull. Izobret.*, No. 25 (1996).
26. A. B. Tomchin, V. S. Velezheva, and A. I. Mel'man, USSR Inventor's Certificate No. 1809609, *Byull. Izobret.*, No. 14 (1993).