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SULFUR-CONTAINING AMINOACID DERIVATIVES OF CHOLCHICINE AND CHOLCHAMINE AND DERIVATIVES OF ISOTHIOURONIUM AND MERCAPTOETHYLAMINE

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New sulfur-containing derivatives of cholchicine and cholchamine with reduced toxicity and preserved pharmacological properties (cytostatic activity for human cancer cells) are prepared. The ability of the new compounds to affect radiation treatment is studied, enabling cholchicine derivatives with more significant radio-sensitizing properties to be found.

The antimitotic and radio-sensitizing properties of cholchicine are responsible for the interest in synthesizing less toxic analogs that surpass the starting compound in activity. We have used sulfur-containing aminoacids and certain aminothiols to modify cholchicine and cholchamine and have measured their ability to affect radiological treatments [1, 2].

The reaction of cholchicine and aminoacids to produce N-cholchicidyl derivatives has been studied by Kiselev [3]. It was noted that the reaction with acidic and monobasic aminoacids occurs only in the presence of base, which is needed to destroy the aminoacid zwitter-ion. A 20-fold excess of aminoacid is used. For basic aminoacids (arginine, lysine, ornithine), the condensation occurs in the presence of a small excess of aminoacid without base. The terminal amino group is substituted.

We used a milder base, potash, to destroy the aminoacid zwitter-ions for the reactions of cholchicine and cholchamine with S-containing aminoacids. The strong base NaOH destroys the S-containing aminoacids and hydrolyzes part of the starting cholchicine and cholchamine. The aminoacids were used in a 2-10-fold excess. The reaction was performed in aqueous alcohol at room temperature for 4-6 d or with heating to 100°C for 6-10 h. The products were isolated by $CHCl_3$ extraction with subsequent purification by column chromatography. The yields under the above conditions were 60-80%.

The following S-containing aminoacid derivatives of cholchicine (**1**) and cholchamine (**2**) were prepared: 10-desmethoxy-10-N-methioninocholchicine (**3**), 10-desmethoxy-10-N-methioninocholchamine (**4**), 10-desmethoxy-10-N-cystinodicholchicine (**5**), 10-desmethoxy-10-N-cystinodicholchamine (**6**), 10-desmethoxy-10-N-cysteinocholchicine (**7**), 10-desmethoxy-10-N-cysteinocholchamine (**8**).

The amino groups of methionine and cystine substitute for the methoxy groups in the tropolone ring of the alkaloids. The condensation with cysteine is expected to occur also at the sulfhydryl group of the aminoacid, which is highly reactive. For example, it has been suggested [4, 5] that the mechanism of action of cholchicine involves binding to SH groups of tubulins, proteins of the mitotic system, or cleavage of S–S bonds of the proteins, thereby disturbing the sulfhydryl—disulfide equilibrium in tubulins [6].

However, sulfhydryl groups were qualitatively observed during the analysis of cysteine derivatives of both cholchicine and cholchamine. Quantitative analysis showed close to 100% of the SH group content in preparations stored for a long time in air. The products from the reaction of cholchicine and cholchamine with cystine do not color a solution of iodine and do not react with nitroprusside. If solutions of the cystine derivatives are treated with sodium sulfite and then nitroprusside, then a red color develops. This is characteristic of the following reactions:

$$R\text{-}S\text{-}S\text{-}R \xrightarrow{Na_2SO_3} R\text{-}SH + R\text{-}S\text{-}SO_3$$

This indicates the presence of disulfides in these compounds.

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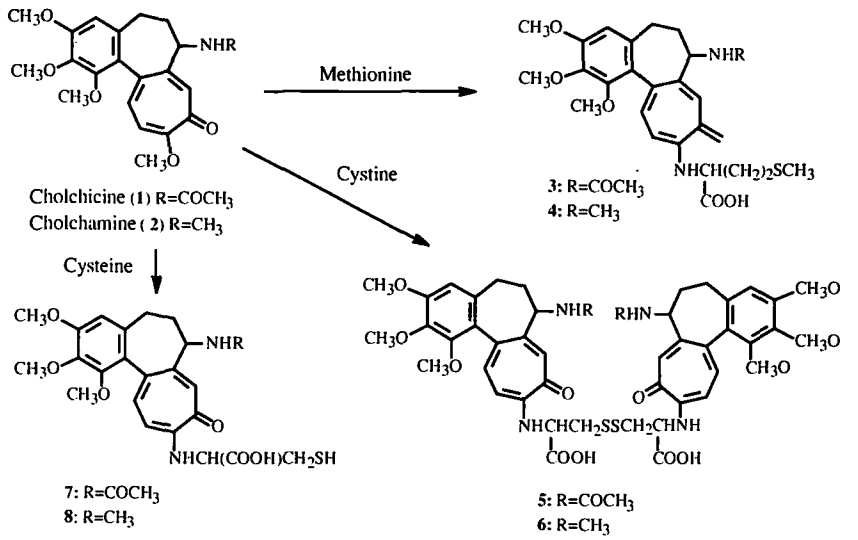
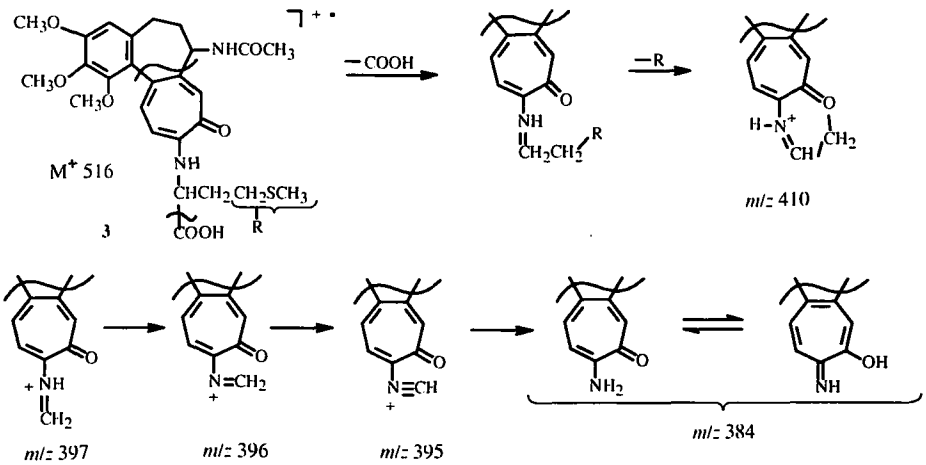


The above data and structure analysis of the cholchicine and cholchamine derivatives (see below) indicate that the amino group of the S-containing aminoacids condenses with the tropolone alkaloids and not the sulfhydryl or disulfide.

UV spectra of the aminoacid derivatives differ from those of the starting alkaloids by the presence of new bands in the range 200-220 nm. Furthermore, the bands at 350 nm undergo bathochromic shifts of 10-15 nm and shoulders appear at 410-415 nm, most prominently for the methionine derivatives. A redistribution of electron density after introducing new electron-donor substituents into the molecule explains these phenomena. Such changes in the UV spectra are characteristic of amino derivatives of cholchicine and are due to the presence of a conjugated N atom in the structure [7].

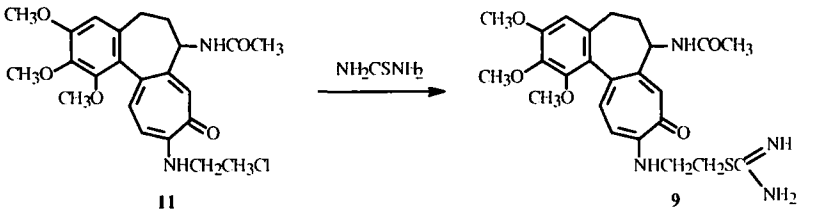
The PMR spectra of the derivatives do not contain a singlet at 4.0 ppm for the methoxy group on the tropolone ring. The 3-proton singlets at 3.6-3.8 ppm for the three methoxy groups on the benzene and the signals for the N–$COCH_3$ or N-CH_3 in the 7-position persist. The pattern of signals at 6.5-7.6 ppm for the aromatic protons of the benzene and tropolone rings undergoes no significant changes. Additional signals for the aliphatic methylene and methine protons of the aminoacids appear at strong field.



The mass spectra of the derivatives, as a rule, contain no peak of the molecular ion owing to the poor volatility. Only for the methionine and cysteine derivatives of cholchicine can weak peaks of the molecular ions (M^+ 516 and 488) be detected. Decarboxylation of the molecular ion with simultaneous cleavage of the bond (C–C or C–S) β to the aminoacid N atom to form

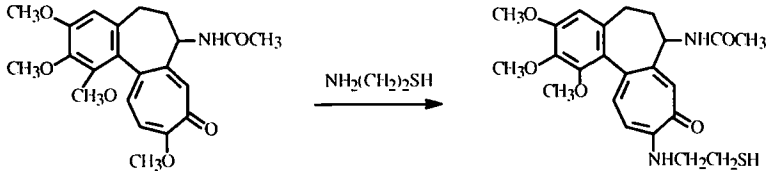
a stable fragment with *m/z* 410 or 411 (cholchicine derivatives) and 382 (cholchamine derivatives) is typical. Fragmentation at the α-, β-, or C–C- bond of the aminoacid residue gives species with successive elimination of two H atoms per lost radical, resulting in fragments with *m/z* 395 (cholchicine derivatives) and 367 (cholchamine derivatives). Finally, mass spectra of the aminoacid derivatives contain very strong peaks (often the base peaks) of pseudomolecular ions of 10-desmethoxy-10-aminocholchicine or 10-desmethoxy-10-aminocholchamine with *m/z* 384 (356). Thus, the positive charge in the molecular ions of the cholchicine and cholchamine S-containing aminoacid derivatives is localized on the 10-position. This produces the fragmentation described above.

In addition to the S-containing aminoacid derivatives, we also synthesized two compounds with a S atom in the substituent, the hydrochlorides of 10-desmethoxy-10-N-aminoethylthiouronium cholchicine (**9**) and 10-desmethoxy-10-N-mercaptoethylamine (**10**). Compound **9** was prepared by reacting 10-desmethoxy-10-N-β-chloroethylamine (**11**) with thiourea [8].



11 **9**

Compound **10** is formed by hydrolysis of **9** to give a complicated mixture of products and by condensation of cholchicine with mercaptoethylamine.



I **10**

Compounds **9** and **10** exhibit similar changes in the UV spectra. The principal bands undergo bathochromic shifts of 10-15 nm. A shoulder appears at 410-420 nm. The shoulder in **9** increases in intensity and converts to a band at 415 nm.

The biological activity of the synthesized compounds was investigated. Their toxicity and mitotic, radio-modifying, and cytostatic activities were studied. It is known that the kinetics of cholchicine activity must be preserved in its derivatives that are potential antitumor preparations. Inhibition of mitosis by cholchicine causes a sharp increase in the number of cells in metaphase. The mitotic indices of intestinal-fold epithelium at various times after administration of the preparations are listed in Table 1.

Table 1 shows that almost all of the cholchicine derivatives cause the mitotic index to increase already 1 h after administration (by a maximum of 3 times compared with the control, 1.5 times compared with cholchicine). Then the amount of mitoses increases, reaches a maximum 3 h later, and remains constant for 6 h. The exception was aminoethylmercaptocholchicine (**10**), administration of which did not change the mitotic activity of intestinal epithelium.

Compounds of this class, which have 1-2 orders of magnitude less toxicity than cholchicine, with more effective radio-sensitizing properties than cholchicine were selected for further study. The results are listed in Table 2.

Table 2 shows that none of the studied compounds would protect animals from high-energy gamma-radiation if administered beforehand. Most of the compounds act as radio-sensitizers and accelerate death from irradiation, like cholchicine. Cholchicine and cholchamine decrease the average lethal dose by 90 R. The derivatives include two compounds that possess more effective radio-sensitizing properties than the natural alkaloids. These are the isothiouronium and cysteine derivatives of cholchicine. The properties of **7** and **9** were similar in toxicity and dose-change factor (DCF) value.

The cytostatic activity of the cholchicine derivatives was studied at the National Cancer Institute (NCI) of the U.S.A. using 60 human tumor types. The compounds were tested at 5 concentrations with 10-fold dilutions over 48 h. Subcategories (strains of a certain tumor type) of leukemia, non-small-cell lung cancer, colon cancer, CNS, melanoma, ovarian and kidney cancer, and prostate and breast cancer were used.

TABLE 1. Mitotic Index of Animal Small Intestine-Fold Epithelium (in percent) after Administration of Cholchicine Derivatives

Compound	Test time, h					
	1	3	6	12	18	24
1	10.29±0.26	21.76±0.31	35.73±0.26	-	-	-
3	7.39±0.17	20.96±0.55	18.05±0.46	-	-	-
5	7.89±0.18	22.13±0.31	21.08±0.45	-	-	-
7	8.30±0.11	19.65±0.31	19.62±0.18	-	-	-
9	15.88±0.13	16.13±0.42	20.53±0.24	-	8.86±0.21	-
10	5.86±0.11	5.93±0.36	4.71±0.07	-	5.27±0.03	-
2	9.96±0.17	19.93±0.36	31.6±0.62	30.76±0.59	5.45±0.37	5.25±0.12
4	7.69±0.18	20.26±0.32	25.71±0.45	-	-	-
6	8.24±0.20	20.91±0.16	8.94±0.50	-	-	-
8	11.65±0.16	15.16±0.35	12.96±0.41	-	-	-
Control	5.54±0.08	5.56±0.30	6.31±0.28	-	6.62±0.08	-

TABLE 2. Dose—Effect Dependence for Cholchicine and Cholchamine Derivatives (30-day animal survival, dose 1/2 $LD_{16/30}$)

Compound	LD_{50}, mg/kg (C57B1/6 mice)	Lethal dose, x-ray			DCF at $LD_{50/30}$	Survival at 800 R, %	Number of animals
		$LD_{16/30}$	$LD_{50/30}$	$LD_{84/30}$			
1	3.9	630	740(691+792)	865	0.9	50	60
3	300	650	745(662+838)	850	0.9	40	60
5	240	610	715(644+785)	780	0.9	20	60
7	100	560	660(589+739)	770	0.9	20	60
9	100	560	630(571+695)	705	0.9	0	60
10	50	640	710(639+781)	780	0.9	0	50
2	56	640	710(693+723)	800	0.9	20	80
4	1100	650	740(803+682)	840	0.9	0	60
6	700					0	60
8	495	770	830(748+921)	900	1.0	80	60
Control		730	830(798+863)	930	-	70	120

We processed the NCI data by averaging the tumor-growth suppression in each subcategory separately for two concentrations at which the activity was highest (10^{-4}-10^{-5} M). The percent scatter in the growth suppression of each subcategory, which contains several tumor types, is rather large. Most of the subcategories contain a type for which the growth was completely inhibited by the preparations. However, there are less sensitive strains. Therefore, averaging the inhibition of tumor types of a given subcategory (CNS cancer, leukemia, etc.) makes it possible to evaluate the effectiveness of the preparation on tumors of a certain type.

Averaging the data for tumor growth of all subcategories at a certain concentration (Σ) provides an indication of the effect of the preparation on all studied tumors of different types. This is a unique index of the preparation effectiveness at doses of 10^{-4}-10^{-5} M. Often these indices provide direct evidence of the activity of the compounds relative to all studied human tumor strains and characterize the range of action of new compounds.

Compounds **3**, **4**, **6**, **7**, and **9** were studied. They all exhibit high cytostatic activity beginning at a dose of 10^{-6} M, for **5**, at 10^{-8} M. They have definite cytostatic and cytolytic effects (except for **1** at a dose of 10^{-4} M). Compound **4** lyses leukemia tumor cells at doses of 10^{-5} and 10^{-6} M.

Compounds **4** and **7** show the most activity against non-small-cell lung cancer (>90%). The remaining compounds in both administered concentrations inhibit the growth of this subcategory strains by >75%.

TABLE 3. Average Suppression by S-Containing Cholchicine Derivatives on Growth of Various Human Tumor Strains in Subcategories (10^{-4}-10^{-5} M concentrations)

Subcategory (strains of certain tumor types)	3		4		6		7		9	
	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-5}	10^{-4}
1. Leukemia	88.17	91	119.0	114.2	96.67	100.66	91.4	102.2	87.4	122.8
2. N.S.Cl Lung C.	75.25	78.75	89.0	91.78	83.5	80.83	92.9	93.95	78.38	79.78
3. Colon	76.0	79.57	89.14	87.71	106.3	113.83	115.7	123.0	95.43	87.3
4. CNS	84.0	91.4	93.67	93.34	84.33	87.5	91.0	100.0	84.67	93.33
5. Melanoma	71.38	74.75	74.57	74.55	68.63	69.25	80.33	85.17	71.5	83.0
6. Ovarial	60.17	61.83	79.67	81.0	59.16	64.33	80.55	85.17	71.5	83.0
7. Renal	61.38	80.75	74.0	77.87	61.40	66.4	72.38	78.5	70.5	89.86
8. Prostate	59.0	68.1	93.0	95.12	79.5	84.0	95.5	91.5	97.56	101.0
9. Breast	62.14	74.38	74.25	74.25	80.0	88.25	70.58	81.45	62.13	82.88
Σ	71	78	87	87	82	84	88	93	80	90

The cystine (**6**) and cysteine (**7**) derivatives are more active toward colorectal cancer. All compounds inhibit CNS cancer by >90%. The results for **6** are somewhat less. All studied compounds suppress the growth of melanoma by at least 70%. Compounds **4**, **7**, and **9** at a dose of 10^{-4} M are active against ovarian cancer. However, the effectiveness of **9** toward cells of this type at a 10-fold less dose (i.e., 10^{-5} M) is <72%. The average growth inhibition for cells of this tumor is 60-85%.

Compound **9** suppressed the growth of kidney tumor types at a dose of 10^{-4} M to 90%. In general, the compounds inhibit the growth of this tumor and ovarian cancer by 61-80%. Compounds **4**, **7**, and **9** suppress the growth of prostate cancer by 91-100%; **3**, 59-68%; **6**, 80-90%. The response of a breast cancer type to the S-containing cholchicine derivatives is rather varied. The activity of all compounds varies. Growth of the tumor cells is suppressed by 62-88%.

Based on the effectiveness indices of the compounds for all categories, the most active compouds are **7** (Σ = 88-93%) and **9** (Σ = 80-90%). Compound **4** can also be considered to be very active owing to its very low toxicity (1100 mg/kg).

Thus, promising compounds for further in vivo research can be selected from the cholchicine and cholchamine derivatives studied at NCI. They are less toxic in animals at the doses at which they exhibit activity. This makes it possible to determine the advisability of further research on one compound or another.

One feature of the behavior of the compounds in the experiment that relates to their mechanism of action should be mentioned. We believe that compounds that interact with tubulin are more active at lower concentrations (10^{-7}-10^{-6} M) whereas those containing alkylating groups, as a rule, are active at a dose of 10^{-4} M.

EXPERIMENTAL

UV spectra were recorded on a Beckman SF-20 instrument in ethanol; IR spectra, on a UR-10 instrument in mineral oil and KBr pellets; PMR spectra, in a Varian XL-100 instrument in $CDCl_3$; mass spectra, in a MAT-311 instrument with direct sample introduction into the ion source. The author thanks P. B. Terent'ev (MGU) for help with interpreting the mass spectra.

10-Desmethoxy-10-N-methioninocholchicine (3). Cholchicine (1 g) in alcohol (10 ml) and methionine (3.4 g) in an aqueous solution (20 ml) of potash (2.5 g) were mixed and boiled on a water bath for 3 h. The mixture was washed with $CHCl_3$ and acidified with 10% HCl to pH 5. The solution was extracted with $CHCl_3$. The extract was concentrated. Addition of ethylacetate produced a precipitate that was separated, dissolved in a small amount of sodium bicarbonate solution, and precipitated by 10% HCl. The product was then dissolved in $CHCl_3$ and purified on an Al_2O_3 column using $CHCl_3$ and then a $CHCl_3$-alcohol (1:1) mixture as eluent. The solvent was removed. The amorphous solid was crystallized from dry ether. Yield of **3**: 1.03 g, 82%, mp 125°C (dec.), $[\alpha]_D^{20}$ = -166° (*c* 0.473, ethanol). Compound **3** is lemon yellow; very soluble in alcohols, $CHCl_3$, acetone; and poorly soluble in water and ether. It has a characteristic odor.

Found, %: N 5.23. Calc., %: N 5.42. $C_{26}H_{32}N_2O_7S$.

UV spectrum (λ_{max}, nm): 220, 250, 355, 410.

IR spectrum (cm^{-1}): 1610 (CO of the tropolone ring), 3560-3290 (NH + OH groups), 1740 (COOH), 1150, 1110, 1050, and 1020 (tricyclic cholchicine system).

PMR spectrum (δ, ppm, J/Hz): 1.99, 2.07 (6H, 2 s, N–Ac, S–CH_3), 2.2 (2H, m, S–CH_2), 2.6 (4H, 2 br. s, 2 H-5 + 2 H-6), 3.55, 3.87, 3.88 (9H, 3 s, 3×OCH_3), 5.3 [3H, m, –CH(COOH)–CH_2–], 4.2 (1H, br. s, H-7), 6.50 (1H, s, H-4), 7.06 (1H, d, J = 12.0, H-12), 7.68 (1H, d, J = 12, H-11), 7.93 (1H, s, H-8), 8.04 (1H, t, NH).

Mass spectrum (*m/z*, relative intensity): M^+ 516 (7), 472 (100), 411 (60), 395 (35), 385 (80), 369 (40), 366 (30), 357 (50), 342 (35).

10-Desmethoxy-10-N-methioninocholchamine (4). The reaction of cholchamine (1 g) and methionine and the isolation of the product were performed by the method used for **3**. Yield of **4**: 0.97 g, 78%, mp 147° (dec.), $[\alpha]_D^{20}$ = -42° (*c* 0.473, ethanol). Compound **4** is yellow; very soluble in alcohols, $CHCl_3$, acetone, Tween-80, and hot water; poorly soluble in ether. It has a characteristic sharp odor.

Found, %: N 5.55. Calc., %: N 5.73. $C_{25}H_{32}N_2O_6S$.

UV spectrum (λ_{max}, nm): 220, 250, 355, 410.

IR spectrum (cm^{-1}): 1610 (CO of the tropolone ring), 3160-3290 (NH + OH), 1740 (COOH), 1150, 1110, 1050, 1020 (tricyclic cholchicine system).

PMR spectrum (δ, ppm, J/Hz): 1.8 (3H, s, N–CH_3), 2.4 (3H, s, S–CH_3), 2.7-3.8 (4H, 2 m, S–CH_2–CH_2), 2.3-1.8 (4H, 2 br. s, 2 H-5 + 2 H-6), 3.50, 3.80, 3.86 (9H, 3 s, 3×OCH_3), 3.4 (1H, br. s, H-7), 6.47 (1H, s, H-4), 6.59 (1H, d, J = 15.0, H-12), 7.30 (1H, d, J = 15, H-11), 7.48 (1H, s, H-8), 8.08 (1H, t, NH).

Mass spectrum (*m/z*, relative intensity): M^+ 516 (7), 472 (100), 411 (60), 395 (35), 385 (80), 369 (40), 366 (30), 357 (50), 342 (35).

10-Desmethoxy-10-N-cystinodicholchicine (5). A solution of cholchicine (1 g) in alcohol (5 ml) was treated with cystine (1.5 g) dissolved in 5% aqueous potash (10 ml). The mixture was boiled on a water bath for 1 h. Then potash (0.5 g) was added. The mixture was boiled for another 20 h. The mixture was washed with $CHCl_3$ and acidified with 10% HCl to pH 3-4. The acidic solution was extracted with a $CHCl_3$-ethanol mixture. The extract was dried with potash, concentrated, and purified on an Al_2O_3 column using $CHCl_3$ and then alcohol. Yield of **5**: 0.85 g, 70%, mp 140° (ethylacetate, dec., chars at 185°), $[\alpha]_D^{20}$ = -179° (*c* 0.947, $CHCl_3$). Compound **5** is light yellow; very soluble in alcohols, $CHCl_3$, acetone; moderately soluble in ethylacetate; insoluble in water and ether.

Found, %: N 5.78. Calc., %: N 5.75. $C_{48}H_{54}N_4O_{14}S_2$.

UV spectrum (λ_{max}, nm): 210, 245, 355.

IR spectrum (cm^{-1}): 1615 (CO of the tropolone ring), 3200-3400 (NH + OH), 1680 (COOH), 1230, 1200, 1150, 1110, 1050, 1020 (tricyclic cholchicine system).

PMR spectrum (δ, ppm): 2.0 (6H, s, 2 N–$COCH_3$), 3.8 (4H, m, 2 S–CH_2–), 2.3 (8H, br. s, 4 H-5 + 4 H-6), 3.53, 3.80, 3.82 (18H, 3 s, 2×3×OCH_3), 4.6 (2H, br. s, 2 H-7), 6.5 (2H, s, 2 H-4).

Mass spectrum (*m/z*, relative intensity): 426 (8), 410 (3), 396 (3), 384 (100), 367 (20), 356 (8), 351 (8), 341 (8), 339 (25), 336 (5), 324 (13).

10-Desmethoxy-10-N-cystinodicholchamine (6). The reaction of cholchamine (1 g) and cystine and the isolation of the product were performed by the method used for **5**. Yield of **6**: 0.87 g, 70%, mp 190°C (ethylacetate, dec.), $[\alpha]_D^{20}$ = -158° (*c* 0.947, $CHCl_3$). Compound **6** is light brown; very soluble in alcohols, $CHCl_3$, acetone; poorly soluble in ethylacetate; insoluble in water and ether.

Found, %: N 6.14. Calc., %: N 6.10. $C_{46}H_{54}N_4O_{12}S_2$.

UV spectrum (λ_{max}, nm): 220, 250, 360.

IR spectrum (cm^{-1}): 1620 (CO of the tropolone ring), 3200-3400 (NH + OH), 1740 (COOH), 1200, 1150, 1110, 1050, 1020 (tricyclic cholchicine system).

PMR spectrum (δ, ppm): 1.2 (2H, m, 2 NH), 2.2 (6H, s, 2 N–CH_3), 3.1-3.8 (4H, m, 2 S–CH_2–), 2.2-2.4 (8H, br. s, 4 H-5 + 4 H-6), 3.4, 3.75, 3.80 (18H, 3 s, 2×3×OCH_3), 6.5 (2H, s, 2 H-4).

Mass spectrum (*m/z*, relative intensity): 390 (3), 382 (8), 375 (3), 367 (8), 356 (100), 351 (5), 341 (15), 328 (25), 325 (15), 207 (25).

10-Desmethoxy-10-N-cysteinocholchicine (7). Cholchicine (1 g) in alcohol (5 ml) and cysteine (1 g) in 5% aqueous potash (10 ml) were mixed and boiled on a water bath for 1 h. Then potash (0.5 g) was added. The mixture was boiled for another 10 h. The product was isolated by the method used for **5**. Yield of **7**: 0.87 g, 76%, mp 188-190°C (ethylacetate),

$[\alpha]_D^{20}$ = -175° (*c* 0.655, $CHCl_3$). Compound **7** is yellowish-brown; very soluble in alcohols, $CHCl_3$, acetone; moderately soluble in ethylacetate; insoluble in water and ether.

The reaction with sodium nitroprusside for an SH group was positive.

Found, %: N 6.01. Calc., %: N 5.74. $C_{24}H_{28}N_2O_7S$.

UV spectrum (λ_{max}, nm): 220, 355.

IR spectrum (cm^{-1}): 3200-3500 (br., NH), 1710 (COOH), 1650 (CO of tropolone), 1230, 1200, 1150, 1110, 1050, 1020 (tricyclic cholchicine system).

PMR spectrum (δ, ppm, J/Hz): 1.2 (1H, SH), 1.90 (3H, br. s, N–Ac), 2.2 (2H, m, S–CH_2), 2.6 (4H, 2 br. s, 2 H-5 + 2 H-6), 3.58, 3.85, 3.88 (9H, 3 s, 3 OCH_3), 3.95 [1H, s, –CH(COOH)–CH_2–], 4.6 (1H, br. s, H-7), 6.48 (1H, s, H-4), 6.82 (1H, d, J = 27, H-12), 7.28 (1H, d, J = 27, H-11), 7.5 (1H, s, H-8).

Mass spectrum (*m/z*, relative intensity): M 488 (3), 426 (6), 410 (5), 409 (3), 384 (100).

Desmethoxy-10-N-cysteinocholchamine (8). The reaction of cholchamine (1 g) and cysteine and the isolation of the product were performed by the method used for **7**. Yield of **8**: 0.77 g, 62%, mp 205-207°C (ethylacetate:ether), $[\alpha]_D^{20}$ = -200° (*c* 0.250, ethanol). Compound **8** is light brown; very soluble in alcohols, $CHCl_3$, acetone; moderately soluble in ethylacetate; insoluble in water and ether.

Found, %: N 5.88. Calc., %: N 6.09; $C_{23}H_{28}N_2O_6S$.

UV spectrum (λ_{max}, nm): 210, 235, 350.

IR spectrum (cm^{-1}): 1600 (CO of the tropolone ring), 3200-3400 (NH + OH), 1740 (COOH), 1200, 1150, 1110, 1050, 1020 (tricyclic cholchicine system).

PMR spectrum (δ, ppm): 1.20 (1H, SH), 1.83 (3H, br. s, N–Ac), 2.40 (2H, m, S–CH_2), 2.50 (4H, 2 s, 2 H-5 + 2 H-6), 3.50, 3.78, 3.82 (9H, 3 s, 3 OCH_3), 6.52 (1H, s, H-4), 7.65 (1H, s, H-8).

10-Desmethoxy-10-N-aminoethylthiouronium cholchicine chlorohydrate (9). 10-Desmethoxy-10-N-chloroethylaminocholchicine chlorohydrate (**11**, 1 g) [8] in absolute ethanol (12 ml) and thiourea (0.16 g) were mixed and boiled on a water bath for 40 h. The mixture was evaporated and purified on an Al_2O_3 column using $CHCl_3$ and then a $CHCl_3$-alcohol mixture. The solvent was removed. The solid was crystallized from dry ether. Yield of **9**: 0.46 g, 42%. Rechromatography of mixed fractions produced another 0.2 g. Total yield of **9**: 61%, mp 160-162°C, $[\alpha]_D^{20}$ = +210° (*c* 0.2, ethanol). Compound **9** is yellow; very soluble in alcohols, $CHCl_3$, acetone; poorly soluble in water and ether.

Found, %: N 12.06. Calc., %: N 12.26. $C_{24}H_{32}N_4O_5SCl_2$.

UV spectrum (λ_{max}, nm): 205, 240, 350, 415.

PMR spectrum (δ, ppm, J/Hz): 2.0 (3H, br. s, N–Ac), 2.2 + 2.6 (4H, m, S–$2CH_2$ + 4H, m, 2 H-5 + 2 H-6), 3.54, 3.84, 3.88 (9H, 3 s, 3 OCH_3), 4.55 (1H, s, H-7), 6.60 (1H, s, H-4), 6.76 (1H, d, J = 11, H-12), 7.28 (1H, d, J = 11, H-11), 7.5 (1H, s, H-8).

10-Desmethoxy-10-N-mercaptoethylaminocholchicine (10). A solution of cholchicine (1 g) in alcohol (5 ml) was treated with mercaptoethylamine hydrotartrate (1.7 g) in 5% aqueous NaOH (5 ml). The course of the reaction (at room temperature) was monitored by chromatography. Cholchicine was totally reacted after 20 d. The products were extracted with $CHCl_3$ and separated on an Al_2O_3 column using a $CHCl_3$-alcohol mixture. Yield of **10**: 0.4 g, 36%, mp 124°C, $[\alpha]_D^{20}$ = -195° (*c* 0.033, ethanol). Compound **10** is gray; very soluble in alcohols, $CHCl_3$, acetone; moderately soluble in ethylacetate; insoluble in water and ether.

The reaction with sodium nitroprusside for SH groups was positive.

Found, %: N 6.12. Calc., %: N 6.30. $C_{23}H_{28}N_2O_5S$.

UV spectrum (λ_{max}, nm): 210, 250, 355.

PMR spectrum (δ, ppm): 2.0 (3H, br. s, N–Ac), 2.2 + 2.6 (4H, m, S-$2CH_2$; 4H, m, 2 H-5; 2 H-6), 3.58, 3.84, 3.86 (9H, 3 s, 3 OCH_3), 4.62 (1H, s, H-7).

The mitotic index of the synthesized compounds for intestinal folds [10] was studied using C57B1/6 mice for 1 d. The studied preparation was administered i.p. at a dose of 1/2 LD_{16}.

Radio-sensitizing activity was studied for healthy male C57B1/6 mice (20-22 g mass, 3-4 months old). Preparations (0.2 ml) were administered at doses of 1/2 LD_{16} 15 min before irradiation, which was applied once in the range of lethal doses ($LD_{16/30}$-$LD_{95-99/30}$, 600-900 R). The dose rate was 100 R/min. Lethal doses were calculated using the Litchfield-Wilcockson method. The effect of the compounds on the post-irradiation death of the animals was evaluated using the dose-change factor (DCF), which is the ratio of $LD_{50/30}$ for the experimental and control groups. The animals were observed for 30 d. The

condition of the animals after administering the synthesized compounds was evaluated through parallel experiments, which over one month studied the leucocyte count in the blood pool and the morphological changes in internal organs.

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