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SYNTHESIS OF PENICILLIN DERIVATIVES AND STUDY OF THEIR CYTOTOXIC PROPERTIES

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By the interaction of heterocyclic thiols with sulfoxides of 6,6-dihydro- and 6α-chloropenicillanates, derivatives of 4-heteryldithio-2-azetidinones have been synthesized, as well as products of their cyclization to form 2β-heterylthiomethyl-and 2β-halomethyl-substituted penicillanates and an ester of 3-chloro-3-methyl-7α-chlorocepham-4-carboxylic acid. Also, the desulfurization of 6α-chloropenicillanate by Raney nickel has been accomplished. For the substances that have been synthesized, a direct relationship has been established between the intensity of their cytotoxic action *in vitro* with respect to tumor cells and the influence of these compounds on the intracellular generation of nitric oxide radicals.

Previously, in the example of sulfones of 7α-chloro- and 7α-methoxycephalosporanates, we had discovered that these compounds are capable of suppressing the *in vitro* growth of various types of tumor cells; we also observed simultaneous, intense intracellular generation of nitric oxide radicals [1]. We are now reporting on an analogous study in which the main objects of investigation were 4-heteryldithio-substituted azetidinones, formed by the interaction of heterocyclic thiols with sulfoxides of 6,6-dihydro- and 6α-chloropenicillanates, as well as products of their cyclization to form the corresponding 2β-heterylthiomethyl- and 2β-halomethyl-substituted penicillanates.

Procedures described in [2-4] were used to accomplish the thermal cleavage of the thiazolidine ring in sulfoxides of esters of penicillanic acids I to form intermediate sulfenic acids that enter into reaction with 2-mercaptobenzothiazole (II*a*, BT) or 1-methyl-2-mercaptoimidazole (II*b*, MI), to obtain the isomeric 4-heteryldithio-substituted azetidinones III and IV (see Scheme 1).

Analysis of the structure of the reaction products demonstrated that the benzothiazole heterocyclic system influences the preferential formation of only certain isopropenyl isomers of azetidinone III*a-d*. The isomerization of III*a* to the isopropylidene-substituted IV*a* was accomplished by the use of aluminum oxide.

The analogous reaction of I with II*b* leads to the formation of a complex mixture consisting of the isomeric azetidinones III*e-g* and IV*b-d* and the penicillanates V*a,b*. The ratio of isomeric azetidinones containing this heterocycle was established by means of HPLC (Table 1). From these data it can be seen that the ratio of isomers depends on the presence or absence of a chlorine atom in position 3 of the azetidinone and on the nature of the ester group.

TABLE 1. Ratio of Isomeric 4-(1-Methylimidazolyl-2)-dithioazetidinones III and IV Recovered from Reaction Mixture

Initial penicillin	R	R^1	Ratio III/IV
Ia	H	$CHPh_2$	90 : 10 (IIIe/IVb)
Ib	Cl	$CHPh_2$	9 : 91 (IIIf-IVc)
Ic	Cl	*t*-Bu	85 : 15 (IIIg/IVd)

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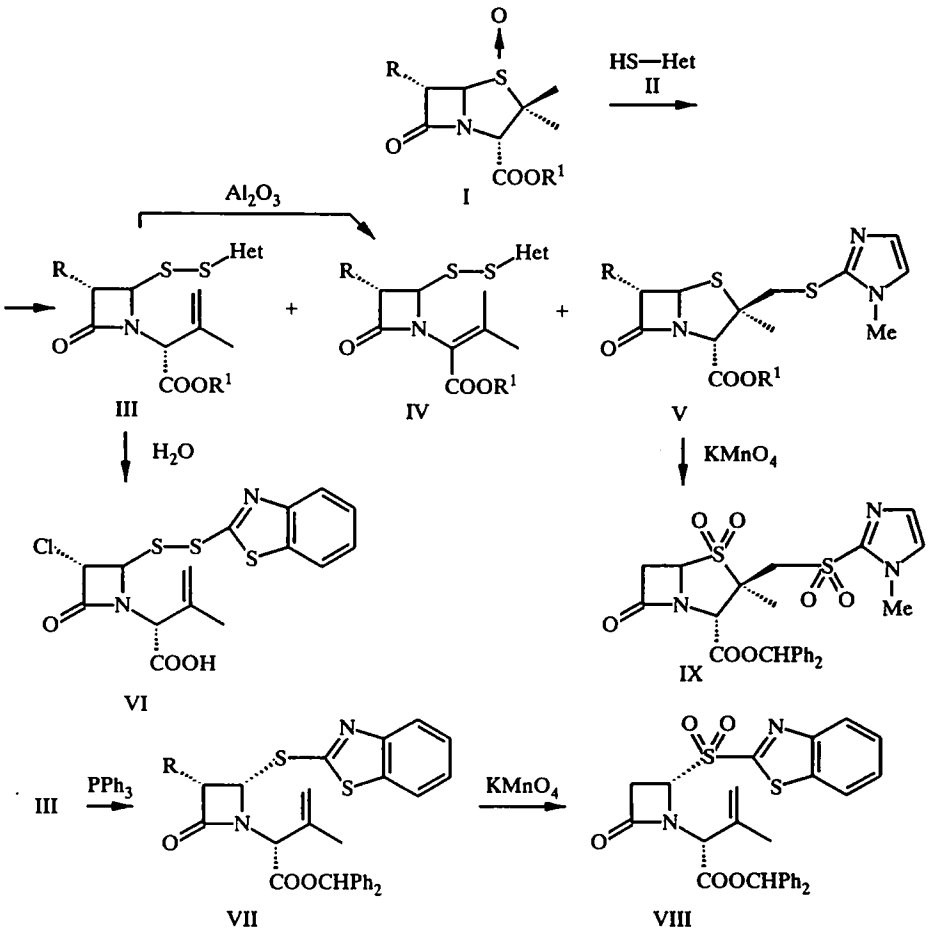
Scheme 1



I a R = H, R^1 = $CHPh_2$; b R = Cl, R^1 = $CHPh_2$; c R = Cl, R^1 = *t*-Bu; dR = Cl, R^1 = $SiMe_3$; II a Het = benzothiazolyl-2 (BT), b Het = 1-methylimidazolyl-2 (MI); III a R = H, R^1 = $CHPh_2$ Het = BT; b R = Cl, R^1 = $CHPh_2$, Het = BT; c R = Cl, R^1 = *t*-Bu, Het = BT; dR = Cl, R^1 = $SiMe_3$, Het = BT; eR = H, R^1 = $CHPh_2$, Het = MI; fR = Cl, R^1 = $CHPh_2$, Het = MI; g: R = Cl, R^1 = *t*-Bu, Het = MI; IV a R = H, R^1 = $CHPh_2$ Het = BT; b R = H, R^1 = $CHPh_2$, Het = MI; c R = Cl, R^1 = $CHPh_2$, Het = MI; dR = Cl, R^1 = *t*-Bu, Het = MI; V a R = H, R^1 = $CHPh_2$; b R = Cl, R^1 = *t*-Bu; VII a R = H, bR = Cl

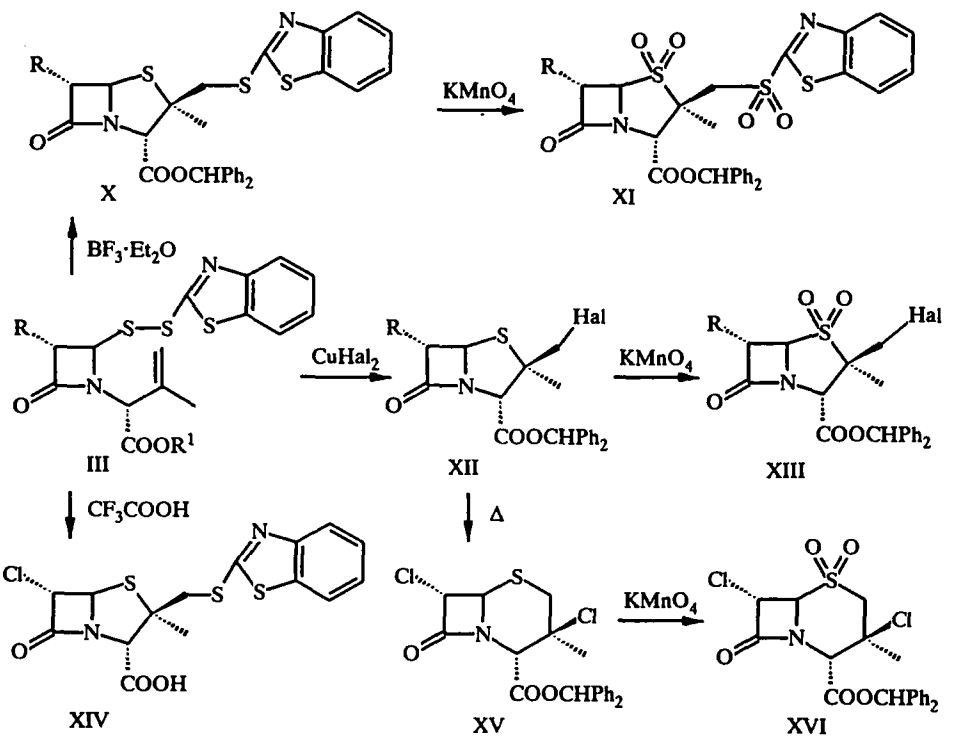
Compound VI, a 4-(benzothiazolyl-2)dithio-substituted azetidinone with a free carboxyl group, was obtained as a result of hydrolytic cleavage of the Si–O bond in III*d*.

4-Monothio-substituted azetidinones VII were obtained by desulfurization of III*a,b* by triphenylphosphine, following a procedure given in [5]. In the case of VII*b*, removal of the sulfur atom was accompanied by a change in configuration of the substituents at the $C_{(4)}$ atom.

The sulfones VIII and IX were synthesized by oxidation of the thio group in VII*a* and V*a* by potassium permanganate. Repeated attempts that were made to oxidize VII*b* by the action of potassium permanganate, and also by the action of hydrogen peroxide and *m*-chlorobenzoic acid, resulted in decomposition of the β-lactam ring in VII*b*.

In Scheme 2, we show reactions based on intramolecular closure of the thiazolidine ring in esters of 4-(benzothiazolyl-2)dithio-substituted azetidinones, accompanied by functionalization of the 2β-methyl group [3, 6]. Cyclization of the azetidinones III to 2β-(benzothiazolyl-2-thio)methylpenicillanates X was accomplished by the use of boron trifluoride etherate, and cyclization to 2β-halomethylpenicillanates XII was accomplished by the use of copper halides. Treatment of III*c* with trifluoroacetic acid to split off the *tert*-butyl group favored the formation of 6α-chloro-2β-(benzothiazolyl-2-thio)methylpenicillanic acid (XIV). Thermal treatment of XII*b* favored its isomerization

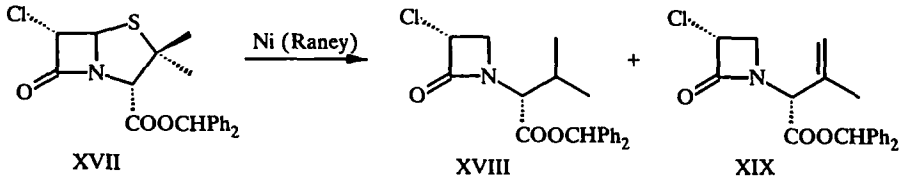
Scheme 2



III a R = H, R^1 = $CHPh_2$; b R = Cl, R^1 = $CHPh_2$; c R = Cl, R^1 = *t*-Bu; X, XI a R = H, b R = Cl; XII, XIII a R = H, Hal = Cl; b R = Cl, Hal = Cl; c R = Cl, Hal = Br

to the benzhydryl ester of 6α-chloro-3-methyl-3-chlorocepham-4-carboxylic acid (XV). Analogs of penicillin and cephalosporin X, XII, and XV were oxidized to the corresponding sulfones XI, XIII, and XVI by potassium permanganate in a mixture of acetic acid and water.

Desulfurization of the 6α-chloropenicillanate XVII, by analogy with a method proposed in [7], led to the formation of a 2:1 mixture of the azetidinones XVIII and XIX, which could not be separated.



The biological part of this study included the determination of cytotoxic properties of the synthesized products relative to tumor and normal cells, and also the ability of these compounds to generate intracellular synthesis of nitric oxide radicals, which are known to be a cause of cell death when present in high concentrations [8].

The concentrations of substances giving a 50% death rate of cells *in vitro* (TD_{50}) were determined by a standard procedure [9] on four lines of tumor cells: NT-1080 (human fibrosarcoma), MG-22A (mouse hepatoma), B16 (mouse melanoma) and Neuro 2A (mouse neuroblastoma), and also on Fibroblasts (normal cells of mouse fibroblast).

The synthesized compounds can be divided into three groups depending on the biological effect that is manifested. The first group consists of substances that do not have any cytotoxic properties at concentrations of 100

TABLE 2. Biological Properties of Synthesized Compounds

Compound	Cytotoxic effect (μg/ml) and specific NO-generating capability in relation to tumor cells					
	MG-22A			HT-1080		
	TD_{50} (CV)*	TD_{50} (MTT)*2	TG_{100}*3	TD_{50} (CV)	TD_{50} (MTT)	TG_{100}
VIIb	>100	>100	12	>100	>100	16
IX	>100	>100	18	>100	>100	22
XIb	100	>100	61	>100	>100	41
XVI	100	>100	26	>100	>100	29
XIIIa	100	53	800	>100	90	142
XIa	50	50	850	50	50	243
XIIIb	13	46	1100	40	42	467
XIIIc	9	42	800	45	34	797
IIIb	27	38	1100	41	45	498
IIIg	18	29	400	35	35	104
VI	44	44	750	55	48	97
Mixture XVIII and XIX	40	53	600	46	55	99

*Concentration giving 50% death of cells (CV = crystal violet stain).
*2Concentration giving 50% death of cells (MTT = 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide stain).
*3Specific NO-generating capability.

μg/ml or lower (see Table 2). Placed in this group is the monothio-substituted azetidinone VII*b*; sulfones of the 2β-(1-methylimidazolyl-2-thio)- and 2β-(benzothiazolyl-2-thio)methylpenicillanates IX and XI*b*; the 3,6-dichlorocephalosporanate XVI; and the sulfone of 2β-chloromethyl-6,6-dihydropenicillanate XIII*a*.

The second group consists of compounds that manifest a moderate cytotoxic effect (see Table 2), namely the sulfones of 2β-(benzothiazolyl-2-thio)methyl-6,6-dihydropenicillanate XI*a* and 2β-halomethylpenicillanates XIII*b,c*, along with certain structural types of azetidinones.

All of the compounds in the third group (see Table 3), i.e., the most active substances, are azetidinones. Their cytotoxic action *in vitro* extends over a broad range of tumor cells. For all of the compounds except VIII, application at a concentration of 10 μg/ml (toxic for most of the tumors) did not suppress the growth of normal cells of the Fibroblasts, thus indicating that these compounds are selective in their toxicity.

For all three groups of substances, we find a correlation between the cytotoxic concentrations and the intensity of intracellular generation of nitric oxide radicals, indicating an interrelation of these two biological effects and hence the good prospects of finding new cytotoxic substances among the esters of 2-[4-(heteryldithio)-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid.

EXPERIMENTAL

PMR spectra were taken in a Bruker WH-90/DS spectrometer (90 MHz) in $CDCl_3$, internal standard TMS. Elemental analyses were performed by means of a Carlo Erba 1108 analyzer. HPLC data were obtained in a Du Pont Model 8800 instrument equipped with a UV detector (λ = 254 nm) and a column (4.6×250 mm) packed with Symmetry C_{18} phase, in a system consisting of acetonitrile and 0.1 M phosphate buffer with pH 2.5 (60:40 ratio), throughput rate 0.8-1.5 ml/min.

TABLE 3. Cytotoxic Effect (μg/ml) and Specific NO-Generating Capability in Relation to Tumor and Normal Cells

Compound	MG-22A			HT-1080			B16			Neuro 2A			Fibroblasts
	TD_{50} (CV)*	TD_{50} (MTT)*2	TG_{100}*3	TD_{50} (CV)	TD_{50} (MTT)	TG_{100}	TD_{50} (CV)	TD_{50} (MTT)	TG_{100}	TD_{50} (CV)	TD_{50} (MTT)	TG_{100}	TD_{50} (CV)
IIIa	3,5	7,0	1350	6,0	10	910	5,0	3,8	625	80	>100	26	>10
IIIc	3,2	4,0	700	5,1	5,4	850	5,7	7,6	621	53	54	850	>10
IIIe	8,0	6,0	750	31	7,0	555	8,0	28	750	52	53	750	>10
VIIa	5,0	5,0	1350	6,0	7,0	1450	1,5	2,2	100	>100	2,0	28	>10
VIIb	4,5	4,0	650	6,0	7,0	633	32	49	1100	49	56	800	>10
VIII	4,5	4,1	1150	4,1	4,6	1350	3,2	4,3	674	6,4	7,1	900	1,0

*Concentration giving 50% death of cells (CV = crystal violet stain).
*2Concentration giving 50% death of cells (MTT = 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide stain).
*3Specific NO-generating capability.

The course of the reaction was monitored by TLC on Merck Kieselgel plates, with UV development. The preparative column chromatography was performed with silica gel, Merck Kieselgel (0.063-0.230 mm). Reagents and materials from Aldrich, Acros, and Sigma were used in the experiments.

The **benzhydryl ester of 2-[4-(2-benzothiazolyldithio)-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid (IIIa)** and the **benzhydryl ester of 2-[4-(2-benzothiazolyldithio)-3-chloro-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid (IIIb)** were synthesized by procedures given in [2, 3].

***tert*-Butyl Ester of 2-[4-(1-Benzothiazolyldithio)-3-chloro-2-oxoazetidinyl-1]-2-(isopropenyl)acetic Acid (IIIc).** To a solution of the *tert*-butyl ester of 6-aminopenicillanic acid 1.7 g (6.26 mmoles) in 23 ml of dichloromethane, there were added isopropyl nitrite 0.78 ml (7.5 mmoles) and three drops of trifluoroacetic acid, after which the solution was stirred for 30 min at 30°C, checking for completion of the reaction as indicated by disappearance of the spot for the original amino acid with R_f 0.31 and the appearance of a new spot with R_f 0.71 in TLC in a hexane–ethyl acetate system, 1:1. The solvent was evaporated under reduced pressure. The crystalline *tert*-butyl ester of 6-diazopenicillanic acid (IR spectrum 2980, 2940, 2080, 1770, 1740 cm^{-1}) was dissolved in 20 ml of dichloromethane; to the resulting solution, 2 ml of ethyl acetate saturated with hydrogen chloride (2 M solution) was added. The mixture was stirred for 20 min at room temperature, and the solvent was evaporated under reduced pressure. The residue was fractionated in a chromatographic column with silica gel (eluent hexane–ethyl acetate, 2:1). The fractions with R_f 0.63 were combined and evaporated down, obtaining 900 mg (49%) of the *tert*-butyl ester of 6α-chloropenicillanic acid. PMR spectrum ($CDCl_3$): 1.53 (9H, s, *t*-Bu); 1.55 (3H, s, CH_3); 1.61 (3H, s, CH_3); 4.44 (1H, s, C_3-H); 4.77 (1H, d, J = 1, C_5-H); 5.33 (1H, d, J = 1, C_6-H).

To a solution of the *tert*-butyl ester of 6α-chloropenicillanic acid 200 mg (0.69 mmole) in 15 ml of dichloromethane, at 0°C, *m*-chloroperbenzoic acid 118 mg (0.69 mmole) was added, and then two additional portions of this acid, 33 mg each, at intervals of 10 min. The mixture was stirred for 1 h at room temperature, diluted with 80 ml of dichloromethane, washed with a 5% Na_2SO_3 solution (30 ml) and a 5% Na_2CO_3 solution (2×30) ml), and dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was fractionated in a chromatographic column with silica gel (eluent ethyl acetate–hexane, 1:2). The fractions with R_f 0.17 were combined and evaporated down, obtaining 201 mg (95%) of the *tert*-butyl ester of the sulfoxide of 6α-chloropenicillanic acid, in the form of a mixture of the 1*S* (90%) and 1*R* (9%) isomers, as indicated by HPLC data. PMR spectrum ($CDCl_3$) of 1*S* isomer: 1.26 (3H, s, CH_3); 1.51 (9H, s, *t*-Bu); 1.66 (3H, s, CH_3); 4.44 (1H, s, C_3-H); 4.97 (1H, d, J = 1, C_5-H); 5.06 (1H, d, J = 1, C_6-H); PMR spectrum ($CDCl_3$) of 1*R* isomer: 1.26 (3H, s, CH_3); 1.47 (9H, s, *t*-Bu); 1.66 (3H, s, CH_3); 4.37 (1H, s, C_3-H); 4.77 (1H, d, J = 2, C_5-H); 5.05 (1H, d, J = 2, C_6-H). IR spectrum (white mineral oil): 1810, 1740, 1050 (S=O) cm^{-1}.

A solution of the *tert*-butyl ester of the sulfoxide of 6α-chloropenicillanic acid 500 mg (1.63 mmoles) and 2-mercaptobenzothiazole 272 mg (1.63 mmoles) in 20 ml of dry toluene was refluxed 2 h, checking for completion of the reaction by means of TLC in a system of hexane–ethyl acetate 1:1. The reaction mixture was cooled, and the toluene was evaporated under reduced pressure. The residue was chromatographed in a column with silica gel (eluent hexane–ethyl acetate, 2:1). The fractions with R_f 0.50 were combined and evaporated down, obtaining 300 mg (26%) of a substance with mp 73-74°C. PMR spectrum ($CDCl_3$): 1.46 (9H, s, *t*-Bu); 1.93 (3H, s, CH_3); 4.73 (1H, s, NCHCOO); 5.04 (1H, d, J = 1, C_4-H); 5.13, 5.20 (2H, 2 br.s, C = CH_2); 5.26 (1H, d, J = 1, C_3-H); 7.33-8.02 (4H, m, C_6H_4). Found, %: C 50.29; H 4.66; N 5.94. $C_{19}H_{21}ClN_2O_3S_3$. Calculated, %: C 49.93; H 4.62; N 6.13.

Benzhydryl Ester of 2-[4-(1-Methylimidazolyl-2-dithio)-2-oxoazetidinyl-1]-2-(isopropenyl)acetic Acid (IIIe). A suspension of the benzhydryl ester of the sulfoxide of 6,6-dihydropenicillanic acid 200 mg (0.50 mmole), 2-mercapto-1-methylimidazole 60 mg (0.50 mmole), and Al_2O_3 500 mg in 6 ml of dry toluene was refluxed 1 h, checking for completion of the reaction by means of TLC in a system of chloroform–acetone, 9:1. The reaction mixture was cooled, then filtered through a layer of silica gel, and the toluene was evaporated under reduced pressure. The residue was chromatographed in a column with silica gel (eluent chloroform–acetone, 9:1). The fractions with R_f 0.38 were combined and evaporated down, obtaining 52 mg (21%) of a mixture which, according to HPLC data, consisted 90% of the benzhydryl ester of 2-[4-(1-methylimidazolyl-2-dithio)-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid (IIIe). PMR spectrum ($CDCl_3$): 1.82 (3H, s, CH_3); 3.31-3.46 (2H, m C_3-H_2); 3.68 (3H, s, NCH_3); 4.84 (1H, s, NCHOO); 4.84 (1H, d, J = 1, =CH_2); 5.04 (1H, d, J = 1, =CH_2); 5.22-5.35 (1H, m, C_4-H); 6.93 (1H, s, $CHPh_2$); 6.95 (1H, d, J = 0.5, imidazole); 7.06 (1H, d, J = 0.5, imidazole); 7.33 (10H, s, $2C_6H_5$). Found, %: C 63.49; H 5.61; N 8.13. $C_{25}H_{25}N_3O_5S_2 \cdot 0.25C_6H_{14}$. Calculated, %: C 63.51; H 5.49; N 8.38.

The mixture's content of the benzhydryl ester of 2-[4-(1-methylimidiazolyl-2-dithio)-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid (IV*b*), according to HPLC data, was 10%. The PMR spectrum exhibited the characteristic signals of methyl groups: 2.04 (3H, s, CH_3); 2.24 (3H, s, CH_3); 3.66 (3H, s, N-CH_3).

***tert*-Butyl Ester of 2-[4-(1-Methylimidazolyl-2-dithio)-3-chloro-2-oxoazetidinyl-1]-2-(isopropenyl)acetic Acid (IIIg).** A solution of the *tert*-butyl ester of the sulfoxide of 6α-chloropenicillanic acid 300 mg (0.98 mmole) and 2-mercapto-1-methylimidazole 137 mg (1.20 mmoles) in 20 ml of dry toluene was refluxed 2 h, checking for completion of the reaction by means of TLC in a system of hexane–ethyl acetate, 1:1. The reaction mixture was cooled, and the toluene was evaporated under reduced pressure. The residue was chromatographed in a column with silica gel (eluent hexane–ethyl acetate 2:1, then hexane–ethyl acetate 1:1). The fractions with R_f 0.23 were combined and evaporated down, obtaining 40 mg (10%) of a mixture containing, according to HPLC data, 85% *tert*-butyl ester of 2-[4-(1-methylimidazolyl-2-dithio)-3-chloro-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid (IIIg). PMR spectrum ($CDCl_3$): 1.46 (9H, s, *t*-Bu); 1.80 (3H, s, CH_3); 3.77 (3H, s, N-CH_3); 4.62 (1H, s, CHCOO); 5.06, 5.12 (2H, 2 s, C=CH_2); 5.15 (H, d, J = 1, C_4-H); 5.71 (1H, d, J = 1, C_3-H); 7.00, 7.08 (2H, dd, J = 0.5 imidazole).

The mixture's content of the *tert*-butyl ester of 2-[4-(1-methylimidazolyl-2-dithio(-3-chloro-2-oxoazetidinyl-1]-2-(isopropylidene)acetic acid (IV*d*), according to HPLC data, was 15%. The PMR spectrum exhibited the characteristic signals of methyl groups: 2.02 (3H, s, CH_3); 2.22 (3H, s, CH_3); 3.64 (3H, s, N-CH_3).

Benzhydryl Ester of 2-[4-(2-Benzothiazolyldithio)-2-oxoazetidinyl-1]-2-(isopropylidene)acetic Acid (IV*a*). To a solution of the benzhydryl ester of 2-[4-(2-benzothiazolyldithio)-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid 200 mg (0.37 mmole) in 10 ml of dichloromethane, Al_2O_3 100 mg was added. The suspension was stirred 48 h at room temperature and then filtered; the filtrate was evaporated under reduced pressure. The residue was chromatographed in a column with silica gel (eluent dichloromethane–acetone, 60:1), obtaining 124 mg (62%) of a substance with mp 45-46°C and with a 97% content of the principal substance, according to HPLC data. PMR spectrum ($CDCl_3$): 1.97 (3H, s, CH_3); 2.12 (3H, s, CH_3); 3.20, 3.31 (2H, dd, J = 2, J = 4, C_3-H_2); 5.22, 5.26 (1H, dd, J = 2, J = 4, C_4-H); 6.84 (1H, s, $CHPh_2$); 7.10-7.95 (14H, m, $2C_6H_5$, C_6H_4).

Benzhydryl Ester of 2-[4-(1-Methylimidazolyl-2-dithio)-3-chloro-2-oxoazetidinyl-1]-2-(isopropylidene)acetic Acid (IV*c*). A solution of the benzhydryl ester of the sulfoxide of 6α-chloropenicillanic acid 600 mg (1.44 mmoles) and 2-mercapto-1-methylimidazole 180 mg (1.58 mmoles) in 15 ml of dry toluene was refluxed 4 h, checking for completion of the reaction by means of TLC in a system of ethyl acetate–heptane (3:2). The reaction mixture was cooled, and the toluene was evaporated under reduced pressure. The residue was chromatographed in a column with silica gel (eluent ethyl acetate–heptane, 3:2). The fractions with R_f 0.50 were combined and evaporated down, obtaining 225 mg (30%) of a mixture which, according to HPLC data, contained 91% of the benzhydryl ester of 2-[4-(1-methylimidazolyl-2-dithio)-3-chloro-2-oxoazetidinyl-1]-2-(isopropylidene)acetic acid (IV*c*). PMR spectrum ($CDCl_3$): 2.11 (3H, s, CH_3); 2.28 (3H, s, CH_3); 3.68 (3H, s, N-CH_3); 5.15 (H, d, J = 1, C_4-H); 5.78 (1H, d, J = 1, C_3-H); 6.88-7.11 (3H, m, $CHPh_2$, imidazole); 7.33 (10H, m, $2C_6H_5$). Found, %: C 58.61; H 4.82; N 8.23; S 12.37. $C_{25}H_{24}ClN_3O_3S_2$. Calculated, %: C 58.41; H 4.70; N 8.17; S 12.49.

The mixture's content of the isomeric benzhydryl ester of 2-[4-(1-methylimidazolyl-2-dithio)-3-chloro-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid (III*f*), according to HPLC data, was 9%. The PMR spectrum contained characteristic signals of methyl and methylene groups: 1.84 (3H, s, CH_3); 3.68 (3H, s, NCH_3); 5.89, 4.91 (2H, 2 s, C=CH_2).

2-[4-(2-Benzothiazolyldithio)-3-chloro-2-oxoazetidinyl-1]-2-(isopropenyl)acetic Acid (VI). A solution of the sulfoxide of 6α-chloropenicillanic acid 600 mg (2.38 mmoles), N,O-bis(trimethylsilyl)acetamide 0.583 ml (2.38 mmoles), and 2-mercaptobenzothiazole 138 mg (2.38 mmoles) in 15 ml of dry benzene was refluxed 5 h. The mixture was diluted with dichloromethane, washed with water, dried with anhydrous Na_2SO_4, and evaporated to dryness. The residue was chromatographed in a column with silica gel (eluent hexane–ethyl acetate, 1:1). The fractions with R_f 0.05 were combined and evaporated down, obtaining 100 mg (10%) of an oily, hygroscopic substance with an 86% content of the principal substance according to HPLC data. PMR spectrum ($CDCl_3$): 1.95 (3H, s, CH_3); 4.93 (1H, s, CH-COO); 5.06 (1H, d, J = 1, C_2-H); 5.20, 5.26 (2H, 2 br.s, C=CH_2); 5.26 (1H, br.s, C_3-H); 7.28-8.11 (4H, m, C_6H_4); 9.71 (1H, s, COOH).

Benzhydryl Ester of 2β-(1-Methylimidazolyl-2-thio)methyl-2α-methyl-6,6-dihydropenam-3α-carboxylic Acid (V*a*). A suspension of the benzhydryl ester of the sulfoxide of 6,6-dihydropenicillanic acid 383 mg (1.0 mmole) and 2-mercapto-1-methylimidazole 120 mg (1.05 mmoles) in 10 ml of dry toluene was refluxed 3 h. The reaction mixture was cooled and evaporated under reduced pressure. The residue was chromatographed in a column with silica gel (eluent ethyl acetate–hexane, 3:1). The fractions with R_f 0.57 were combined and evaporated down, obtaining 200 mg (41%) of a substance which, without any special purification, was oxidized to the sulfone IX. PMR spectrum ($CDCl_3$): 1.26 (3H, s, CH_3); 3.06 (1H, dd, J = 2, J = 16, C_6-H_{trans}); 3.55 (1H, dd, J = 4, J = 16, C_6-H_{cis}); 3.62

(3H, s, NCH_3); 3.48, 3.71 (2H, AB-q, J = 14, SCH_2); 4.95 (1H, s, C_3-H); 5.31 (1H, dd, J = 2, J = 4, C_5-H); 6.93 (1H, s, $CHPh_2$); 6.88 (1H, d, J = 1 C_4-$H_{imidazole}$); 7.04 (1H, d, J = 1, C_5-$H_{imidazole}$); 7.35 (10H, m, $2C_6H_5$).

***tert*-Butyl ester of 2β-(1-methylimidazolyl-2-thio)methyl-2α-methyl-6α-chloropenam-3α-carboxylic acid (V*b*)** was obtained in the same manner as compound V*a*, from the *tert*-butyl ester of the sulfoxide of 6α-chloropenicillanic acid and 2-mercapto-1-methylimidazole. Yield 25%, mp 124-125°C. PMR spectrum ($CDCl_3$): 1.45 (9H, s, *t*-Bu); 1.51 (3H, s, CH_3); 3.24, 3.57 (2H, AB-q, J = 14, CH_2S); 3.57 (3H, s, NCH_3); 4.68 (1H, s, C_3-H); 4.77 (1H, d, J = 0.5, C_5-H); 5.22 (1H, d, J = 0.5, C_6-H); 6.88 & 7.00 (2H, dd, J = 0.5 imidazole). Found, %: C 47.42; H 5.42; N 10.25. $C_{16}H_{22}ClN_3O_3S_2$. Calculated, %: C 47.58; H 5.49; N 10.40.

Benzhydryl Ester of 2-[4-(2-Benzothiazolylsulfonyl)-2-oxoazetidinyl-1]-2-(isopropenyl)acetic Acid (VIII). A solution of the benzhydryl ester of 2-[4-(2-benzothiazolylthio)-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid (III*a*) 532 mg (1.0 mmole) and triphenylphosphine 265 mg (1.0 mmole) in 1.7 ml of dichloromethane was held 1 h at room temperature. The reaction mixture was diluted with 10 ml of dichloromethane and filtered through a layer of silica gel, after which the solvent was evaporated under reduced pressure. The residue was chromatographed in a column with silica gel (eluent ethyl acetate–heptane, 3:1). The fractions with R_f 0.33 were combined and evaporated down, obtaining 180 mg (36%) of the benzhydryl ester of 2-[4-(2-benzothiazolylthio)-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid (VII*a*), which was used without any special purification in the subsequent reaction. PMR spectrum ($CDCl_3$): 1.75 (3H, s, CH_3); 3.13 (1H, dd, J = 16, J = 2, C_3-H_{trans}); 3.62 (1H, dd, J = 16, J = 5, C_3-H_{cis}); 4.91 & 5.08 (2H, dd, J = 0.5, C = CH_2); 4.97 (1H, s, NCHCOO); 5.73 (1H, dd, J = 2, J = 5, C_4-H); 7.00 (1H, s, $CHPh_2$); 7.35 (10H, s, $2C_6H_5$); 7.57-7.77 (4H, m, C_6H_4).

To a solution of VII*a* 180 mg (0.36 mmole) in 3.0 ml of acetic acid and 0.5 ml of water, $KMnO_4$ 135 mg (0.84 mmole) was added. The resulting suspension was mixed 3 h at room temperature and then chilled to 0°C, after which the excess $KMnO_4$ was reduced by 25% hydrogen peroxide. The solution was filtered, diluted with 30 ml of water, and extracted with 30 ml of dichloromethane. The organic phase was washed with a 7% $NaHCO_3$ solution and dried with anhydrous Na_2SO_4, after which the dichloromethane was evaporated under reduced pressure. The residue was chromatographed in a column with silica gel (eluent ethyl acetate–heptane, 1:3). The fractions with R_f 0.19 were combined and evaporated down, obtaining 60 mg (31%) of amorphous powder with a content of the principal substance 93% according to HPLC data. PMR spectrum ($CDCl_3$): 1.77 (3H, s, CH_3); 3.33 (1H, dd, J = 13, J = 5, C_3-H_{trans}); 3.55 (1H, dd, J = 13, J = 3, C_3-H_{cis}); 4.91 & 5.13 (2H, br.s, br.s, C=CH_2); 5.06 (1H, s, NCHCOO); 5.26 (1H, dd, J = 3, J = 5, C_4-H); 6.84 (1H, s, $CHPh_2$); 7.31 (10H, s, $2C_6H_5$); 7.51-8.22 (4H, m, C_6H_4). Found, %: C 62.99; H 4.63; N 5.04. $C_{28}H_{24}N_2O_5S_2$. Calculated, %: C 63.14; H 4.53; N 5.26.

Benzhydryl ester of 2-[4-(2-benzothiazolylthio)-3-chloro-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid (VII*b*) was obtained in the same manner as compound VII*a*, from the benzhydryl ester of 2-[4-(2-benzothiazolyldithio)-3-chloro-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid (III*b*) and triphenylphosphine. Yield 12%, mp 118-119°C. PMR spectrum ($CDCl_3$): 1.82 (3H, s, CH_3); 5.00 (1H, s, NCHCOO); 5.06 (2H, d, J = 0.5, C=CH_2); 5.24 (1H, d, J = 5, C_4-H); 6.24 (1H, d, J = 5, C_3-H); 7.00 (1H, s, $CHPh_2$); 7.35 (10H, s, $2C_6H_5$); 7.40-7.84 (4H, m, C_6H_4). Found, %: C 62.62; H 4.58; N 4.93. $C_{28}H_{23}ClN_2O_3S_2$. Calculated, %: C 62.85; H 4.33; N 5.24.

Benzhydryl ester of sulfone of 2β-(1-methylimidazolyl-2-sulfonyl)methyl-2α-methyl-6,6-dihydropenam-3α-carboxylic acid (IX) was obtained in the same manner as compound VIII, from the benzhydryl ester of 2β-(1-methylimidazolyl-2-thio)methyl-2α-methyl-6,6-dihydropenam-3α-carboxylic acid. Obtained 74 mg (33%) of a substance with mp 80-84°C, with a content of the principal substance 96% according to HPLC data. PMR spectrum ($CDCl_3$): 1.46 (3H, s, CH_3); 3.48 (2H, m, C_6H_2); 3.91 (3H, s, NCH_3); 3.93, 4.08 (2H, AB-q, J = 14, SCH_2); 4.60 (1H, m, C_5-H) 5.10 (1H, s, C_3-H); 6.93 (1H, s, $CHPH_2$); 6.95 (1H, br.s, imidazole); 7.11 (1H, d, J = 1, imidazole); 7.38 (10H, m, $2C_6H_5$). Found, %: C 55.49; H 5.03; N 7.24. $C_{25}H_{25}N_3O_7S_2 \cdot 0.5CH_3COOC_2H_5$. Calculated, %: C 55.18; H 4.96; N 7.15.

Benzhydryl Ester of Sulfone of 2β-(Benzothiazolyl-2-sulfonyl)methyl-2α-methyl-6,6-dihydropenam-3α-carboxylic Acid (XI*a*). To a solution of the benzhydryl ester of 2-[4-(2-benzothiazolyldithio)-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid (III*a*) 340 mg (0.51 mmole) in 14 ml of a mixture of dichloromethane and nitromethane (1:1), 0.5 ml of $BF_3 \cdot Et_2O$ was added. The reaction mixture was held 24 h at room temperature and evaporated under reduced pressure. The residue of 170 mg (49%) of the benzhydryl ester of 2β-(benzothiazolyl-2-thio)methyl-2α-methyl-6,6-dihydropenam-3α-carboxylic acid (X*a*), without any special purification, was oxidized by $KMnO_4$ in the same manner as compound V*a*. Yield 54%, mp 170-171°C. PMR spectrum ($CDCl_3$): 1.52 (3H, s, CH_3); 3.48 (2H, m, C_6-H_2); 3.90, 4.15 (2H, AB-q, J = 14, CH_2SO_2); 4.59 (1H, dd, J = 2, J = 4, C_5-H); 5.28 (1H, s, C_3-H); 6.85 (1H, s, $CHPh_2$); 7.15-7.44 (10H, m, $2C_6H_5$); 7.48-8.28 (4H, m, C_6H_4). Found, %: C 56.55; H 4.14; N 4.64. $C_{28}H_{24}N_2O_7S_3$. Calculated, %: C 56.36; H 4.05; N 4.68.

Benzhydryl ester of sulfone of 2β-(benzothiazolyl-2-sulfonyl)methyl-2α-methyl-6α-chloropenam-3α-carboxylic acid (XI*b*) was obtained in the same manner as compound XI*a*, from the benzhydryl ester of 2-[4-(2-benzothiazolyldithio)-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid (III*b*). Yield 7%, mp 57-59°C. PMR spectrum ($CDCl_3$): 1.37 (3H, s, CH_3); 3.67, 4.13 (2H, AB-q, J = 14, CH_2S); 5.04 (1H, s, C_3-H); 5.13 (1H, d, J = 1, C_5-H); 5.40 (1H, d, J = 1, C_6-H); 7.00 (1H, s, $CHPh_2$); 7.22-8.00 (14H, m, $2C_6H_5$, C_6H_4). Found, %: C 53.43; H 4.03; N 4.15. $C_{28}H_{23}ClN_2O_7S_3$. Calculated, %: C 53.28; H 3.67; N 4.43.

The benzhydryl ester of the sulfone of 2α-methyl-2β-chloromethyl-6,6-dihydropenam-3α-carboxylic Acid (XIII*a*), the benzhydryl ester of the sulfone of 2α-methyl-2β-chloromethyl-6α-chloropenam-3α-carboxylic acid (XIII*b*), and the benzhydryl ester of the sulfone of 2β-bromomethyl-2α-methyl-6α-chloropenam-3α-carboxylic acid (XIII*c*) were synthesized by a procedure given in [3].

2β-(Benzothiazolyl-2-thio)methyl-2α-methyl-6α-chloropenam-3α-carboxylic Acid (XIV). A solution of the *tert*-butyl ester of 2-[4-(2-benzothiazolyl-2-dithio)-2-oxoazetidinyl-1]-2-(isopropenyl)acetic acid (III*c*) 100 mg (0.21 mmole) in 2 ml of trifluoroacetic acid was held 1 h at room temperature. The solvent was evaporated, and the residue was chromatographed in a column with silica gel (eluent chloroform–ethanol, 2:1). The fractions with R_f 0.50 were combined and evaporated down. Yield 26 mg (32%) with a content of the principal substance 93% according to HPLC data. PMR spectrum ($CDCl_3$): 1.64 (3H, s, CH_3); 3.64, 4.00 (2H, AB-q, J = 14, CH_2S); 4.93 (1H, s, C_3-H); 5.00 (1H, d, J = 0.5, C_5-H); 5.33 (1H, d, J = 0.5, C_6-H); 7.20-7.77 (4H, m, C_6H_4); 9.77 (1H, s, COOH).

Benzhydryl Ester of Sulfone of 3α-Methyl-3β-chloro-7α-chlorocepham-4α-carboxylic Acid (XVI). A mixture consisting of 1 ml of acetonitrile, 1 ml of water, and 170 mg (0.17 mmole) of the benzhydryl ester of 2α-methyl-2β-chloromethyl-6α-chloropenam-3α-carboxylic acid (XII*b*) [3] was heated 8 h at 78-80°C, diluted with 30 ml of water, and washed with 30 ml of dichloromethane. The organic phase was dried with anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The residue was chromatographed in a column with silica gel (eluent ethyl acetate–heptane, 1:2), obtaining 76 mg (44%) of the benzhydryl ester of 3α-methyl-3β-chloro-7α-chlorocepham-4α-carboxylic acid (XV), which, without any special purification, was oxidized by $KMnO_4$ in the same manner as compound V*a*. Yield 54%, mp 159-160°C, with a content of the principal substance 91% according to HPLC data. PMR spectrum ($CDCl_3$): 1.44 (3H, s, CH_3); 3.22, 3.69 (2H, dd, J = 2, J = 15, C_7-H_2); 3.33, 3.77 (2H, dd, J = 15, C_2-H_2); 4.80 (1H, s, C_4-H); 4.89, 4.93 (1H, dd, J = 2, J = 5, C_6-H); 6.95 (1H, s, $CHPh_2$); 7.37 (10H, s, $2C_6H_5$).

Mixture of Benzhydryl Ester of 2-Oxo-3-chloroazetidinyl-1-α-(isopropyl)acetic Acid (XVIII) and Benzhydryl Ester of 2-Oxo-3-chloroazetidinyl-1-α(1-methylethenyl)acetic Acid (XIX). To a solution of the benzhydryl ester of 6α-chloropenicillanic acid 150 mg (0.37 mmole) in 5 ml of ethanol, there was added a quarter teaspoon of freshly prepared Raney nickel in ethanol. The mixture was refluxed 20 min, cooled, and filtered; and the solvent was evaporated under reduced pressure. The residue was chromatographed in a column with silica gel (eluent ethyl acetate–hexane, 1:4). The fractions with R_f 0.22 were combined and evaporated down, obtaining 20 mg (21%) of an oily substance, which, according to HPLC data, contained 66% of the benzhydryl ester of 2-oxo-3-chlorazetidinyl-1-α-(isopropyl)acetic acid (XVIII). PMR spectrum ($CDCl_3$): 0.84 (3H, d, J = 1.5, CH_3); 0.95 (3H, d, J = 1.5, CH_3); 2.0-2.46 (1H, m, $CHMe_2$); 3.15-3.64 (2H, m, C_4-H_2); 6.86 (1H, s, $CHPh_2$); 7.28 (10H, s, $2C_6H_5$). The mixture's content of the benzhydryl ester of 2-oxo-3-chlorazetidinyl-1-α-(1-methylethenyl)acetic acid (XIX), according to HPLC data, was 33%. PMR spectrum ($CDCl_3$): 1.75 (3H, s, CH_3); 3.15-3.64 (2H, m, C_4-H_2); 6.86 (1H, s, $CHPh_2$); 7.28 (10H, s, $2C_6H_5$). (In the PMR spectra, we have listed only those values of the chemical shifts that can provide an unambiguous characterization of the protons entering into the composition of the molecules of XVIII and XIX.)

Biological Tests. The cytotoxic properties of the compounds were studied in cultures of monolayer tumor cells that had been grown in 96 cutout panels in standard medium without indicator or antibiotics, following procedures given in [9]. The numbers of live cells were determined by two independent colorimetric methods on the basis of the intensity of staining of the cell membranes with crystal violet and staining of the mitochrondrial enzymes by 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide, characterizing the intensity of their redox properties.

The specific NO-generating capability of the test substances (TG_{100}), extrapolating this index to 100% live cells, was calculated by means of the equation

$$TG_{100} = G_{EX} \cdot 100/C \ (\text{nmoles} \cdot 10^{-1}/200 \ \mu\text{l})$$

where G_{EX} is the concentration of NO (nmoles) in 200 μl (the volume of a panel cutout) of the culture medium, generated by the live cells after incubation with 50 μg/ml of the test substance, according to a procedure given in

[10]; C is the percentage of live cells after incubation with 50 μg/ml of the test substance, as determined from the intensity of staining of cell membranes by crystal violet [9].

Control cells (without the test substances) were grown in a separate panel.

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