SYNTHESIS AND ANTITUMOR ACTIVITY OF 6-OXO-7-AMINO-5H-PYRIDO[2,3-b]- AND 5H-PYRIMIDO[4,5-b][1,4]THIAZINES

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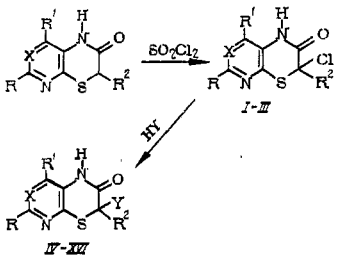
It has been reported [1, 4] that 6-amino- and 6-oxopyrimido[4,5-b] and pyrido[2,3-b][1,4]-thiazines represent a new type of folic antagonist, possessing antitumor activity. Continuing this work, we have synthesized some 6-oxopyrimido[4,5-b]- and 6-oxopyrido[2,3-b][1,4]thiazines with a variety of amino groups at $C_{(7)}$. For this purpose, 4-methoxypyrimidothiazin-6-one-4-methoxy-7-ethoxycarbonylpyrimidothiazin-6-one and 2-chloropyridothiazin-6-one were chlorinated with sulfuryl chloride in the presence of benzoyl peroxide to give the 7-halo compounds (I-III), the structures of which were confirmed by their PMR spectra (the presence of a signal for a proton at $C_{(7)}$ in the case of (I) and (III) (at 3.84 and 6.13 ppm, respectively), and the absence of such a signal for (II) [2]).

These compounds (I-III) are convenient starting materials for the synthesis of a variety of 7-substituted 6-oxopyrido- and 6-oxopyrimidothiazines, especially 7-amino compounds. The reactions with amines were carried out in benzene or aqueous dioxane, using an excess of the amine or Et_3N as an HCl acceptor. Treatment of (I-III) with N-methylpiperazine, morpholine, or piperidine gave the 7-amino derivatives (IV-X):



I-III

IV-XVI

(I): R = R^2 = H, R^1 = OMe, X = N; (II): R = H, R^1 = OMe, R^2 = COOEt, X = N; (III): R = Cl, R^1 = R^2 = H, X = CH; (IV): R = R^2 = H, R^1 = OMe, X = N, Y = N—(N^1-methyl)piperazinyl; (V): R = H, R^1 = OMe R^2 = COOEt, X = N, Y = N-(N^1-methyl)piperazinyl ; (VI): R = Cl, R^1 = R^2 = H, X = CH, Y = N-(N^1-methyl)piperazinyl; (VII): R = R^2 = H, R^1 = OMe, X = N, Y = morpholino; (VIII): R = H, R^1 = OMe, R^2 = COOEt, X = N, Y = morpholino; (IX): R = Cl, R^1 = R^2 = H, X = CH, Y = morpholino; (X): R = Cl, R^1 = R^2 = H, H = CH, Y = piperidino; (XI): R = R^2 = H, R^1 = OMe, X = N, Y = N-pyridinium chloride; (XII): R = R^2 = H, R^1 = OMe, X = N, Y = NHPr-i; (XIII): R = R^2 = H, R^1 = OMe, X = N, Y = p-carboxyphenylamino; (XIV): R = Cl, R^1 = R^2 = H, X = CH, Y = p-carboxyphenylamino; (XV): R = R^2 = H, R^1 = OMe, X = N, Y = $NH(CH_3)C_6H_4CONHCH(COOET)CH_2CH_2COOEt$-p; (XVI): R = R^2 = H, R^1 = OMe, X = N, Y = $NH(CH_3)C_6H_4CONHCH(COOH)CH_2CH_2COOH$-p (Ba salt).

Reaction of (I) with pyridine gave the pyridinium salt (XI). Reaction with aliphatic amines in most instances resulted in resinification, and only in the case of i-$PrNH_2$ was it possible to obtain the 7-isopropyl amino derivative (XII). Reaction of thiazines (I) and (III) with p-aminobenzoic acid gave (XIII) and (XIV), which contain the p-aminobenzoic acid residue. For a comparative study of their biological activity, thiazines (XV) and (XVI) were prepared, which contain the N-methyl-p-aminobenzoyl-L-glutamic acid residue and its diethyl ester, present in the antitumor drug methotrexate. Diethyl N-methyl-p-aminobenzoyl-L-glutamate was obtained as described in [6], and thiazine (I) was reacted with the diethyl ester in aqueous dioxane in the presence of Et_3N.

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A study of the cytostatic activity of the compounds obtained showed that they inhibited the growth of tissue cultures of chick embryo heart and sarcoma 45. In a concentration of 10^{-3} g/ml, the pyridothiazinones (VI), (IX), and (X) inhibited to equal extents the growth of both the normal and culture tissues. The p-aminobenzoic acid derivative (XIV) showed some selectivity towards the tumor tissue. In a concentration of 10^{-3} g/ml it totally suppressed the growth of sarcoma 45 explants, but only inhibited the growth of chick embryo heart tissue culture by 70%. The pyrimidothiazinones (IV), (V), (XI), (XIII), and (XVI) were selective towards the tumor tissue in a concentration of 10^{-3} g/ml, while inhibiting the growth of chick embryo heart by only 45-65%. The greatest cytostatic activity was shown by (VI), (XI), (XIII), (XIV), and (XVI). Comparing the cytostatic activity of 7-aminopyrimido- and pyridothiazinones with their antiblastic activity, only in two instances was it possible to establish a correlation between these properties. Antitumor activity was shown by (VI) *in vivo*. Administration to mice with disseminated sarcoma 180 (daily for 7 days orally in a dose of 150 mg/kg) resulted in 40% inhibition in tumor growth ($\alpha > 0.95$). Antiblastic activity was also shown by (XI). This compound inhibited the growth of transplanted Jensen's sarcoma by 40% ($\alpha > 0.95$), when administered in the same way in a dose of 100 mg/kg. Both compounds were nontoxic, the LD_{95} values following a single dose *per os* in mice being more than 500 mg/kg.

Biological examination of these 7-amino-6-oxopyrimido- and pyridothiazines has therefore shown them to possess antitumor activity in disseminated tumors in animals.

EXPERIMENTAL CHEMISTRY

IR spectra were obtained on a Perkin-Elmer spectrophotometer and a twin-beam UR-10 spectrophotometer. PMR spectra were obtained on an INM-4H-100 instrument, internal standard DMSO. Data on the compounds obtained is given in Table 1.

4-Methoxy-6-oxo-7-chloro-5H-pyrimido[4,5-b][1,4]thiazine (I). To a solution of 1 g (5 mmole) of 4-methoxy-6-oxopyrimido[4,5-b][1,4]thiazine [4] in 30 ml of dry chlorobenzene was added at 70°C 0.1 g (0.4 mmole) of benzoyl peroxide, the mixture brought to boiling (125°C), and 0.7 g (5 mmole) of SO_2Cl_2 in 5 ml of chlorobenzene added over 0.5 h. The mixture was boiled for a further 2.5 h, cooled to 100-110°C, and filtered. The filtrate was cooled to 20°C and the solid which separated was filtered off to give 0.5 g of (I). The chlorobenzene mother liquors were evaporated to dryness *in vacuo*, and the residue triturated with light petroleum to give a further 0.35 g of (I). Colorless crystals, sparingly soluble in benzene, ether, and water, but soluble in alcohol.

4-Methoxy-6-oxo-7-chloro-7-ethoxycarbonyl-5H-pyrimido[4,5-b][1,4]thiazine (II). Similarly, from 2.7 g (10 mmole) of 4-methoxy-6-oxo-7-ethoxycarbonylpyrimido[4,5-b][1,4]thiazine [5] and 11.4 g (10 mmole) of SO_2Cl_2 in the presence of 0.2 g (0.8 mmole) of benzoyl peroxide there was obtained 2.2 g of (II). Colorless crystals, soluble in alcohol and ether.

2-Chloro-6-oxo-7-chloro-5H-pyrido[2,3-b][1, 4]thiazine (III). To a suspension of 1 g (5 mmole) of 2-chloro-6-oxopyrido[2,3-b][1, 4]thiazine [3] and 0.1 g (0.4 mmole) of benzoyl peroxide in 30 ml of dry chlorobenzene was added at 120-125°C over 15-20 min a solution of 0.68 g (5 mmole) of SO_2Cl_2 in 10 ml of chlorobenzene. The mixture was boiled at 125°C for 2 h, cooled, and the solid which separated filtered off to give 0.8 g of (III). The chlorobenzene mother liquors were evaporated to dryness *in vacuo*, and the residue triturated with ether to give 0.2 g of (III).

4-Methoxy-6-oxo-7-N-(N'-methyl)piperazinyl-5H-pyrimido[4,5-b][1,4]thiazine (IV). To a suspension of 0.44 g (1.8 mmole) of (I) in 20 ml of dry benzene was added a solution of 0.38 g (3.6 mmole) of N-methylpiperazine in 5 ml of benzene, and the mixture stirred at 18-20°C for 4 h. The solid which separated was filtered off, washed with water, and dried to give 0.36 g of (IV). The benzene mother liquors were evaporated, the residue washed with water, dried, and triturated with water to give a further 0.15 g of (IV).

4-Methoxy-6-oxo-7-N-(N'-methyl)piperazinyl-7-ethoxycarbonyl-5H-pyrimido[4,5-b][1,4]thiazine (V). To a suspension of 0.5 g (1.6 mmole) of the thiazine (II) in 20 ml of benzene was added a solution of 0.33 g (3.2 mmole) of N-methylpiperazine in 5 ml of benzene. In the same way as for (IV), 0.6 g of (V) was obtained.

2-Chloro-6-oxo-7-N-(N'-methyl)piperazinyl-5H-pyrido[2,3-b][1, 4]thiazine (VI). To a suspension of 0.5 g (2.1 mmole) of the thiazine (III) in 20 ml of dry benzene was added a solution of 0.4 g (4 mmole) of N-methylpiperazine in 10 ml of benzene. In the same way as for (IV), 0.5 g of (VI) was obtained.

4-Methoxy-6-oxo-7-N-morpholino-5H-pyrimido[4,5-b][1, 4]thiazine (VII). To a suspension of 0.5 g (2.1 mmole) of the thiazine (I) in 20 ml of dry benzene was added 0.38 g (4.2 mmole) of morpholine, and the mixture was stirred for 5 h. In the same way as for (IV), 0.58 g of (VII) was obtained.

4-Methoxy-6-oxo-7-N-morpholino-7-ethoxycarbonyl-5H-pyrimido[4,5-b][1, 4]thiazine (VIII). To a suspension of 0.74 g (2.4 mmole) of the thiazine (II) in 30 ml of dry benzene was added 0.43 g (4.8 mmole) of morpholine in 5 ml of benzene. The mixture was stirred for 5 h, and as for (IV), 1.1 g of (VIII) was obtained.

2-Chloro-6-oxo-7-N-morpholino-5H-pyrido[2,3-b][1, 4]thiazine (IX). To a suspension of 1 g (4.2 mmole) of the thiazine (III) in 30 ml of dry benzene was added a solution of 0.74 g (8.4 mmole) of morpholine in 10 ml of benzene, and the mixture stirred for 5 h. This was worked up as for (IV). The solid was filtered off, washed with water, and dried to give 1g of (IX).

2-Chloro-6-oxo-7-N-piperidino-5H-pyrido[2,3-b][1, 4]thiazine (X). To a suspension of 1 g (4.2 mmole) of the thiazine (III) in 30 ml of dry benzene was added a solution of 0.72 g (8.1 mmole) of piperidine in 5 ml of benzene. The mixture was worked up as for (IV) to give 0.83 g of (X).

4-Methoxy-6-oxo-7-N-pyridino-5H-pyrimido[4,5-b][1, 4]thiazine Chloride (XI). A mixture of 0.4 g (1.7 mmole) of (I) in 10 ml of pyridine was stirred at 18-20°C for 7 h. The solid which separated was filtered off and washed with ether to give 0.4 g of (XI).

4-Methoxy-6-oxo-7-isopropylamino-5H-pyrimido[4,5-b][1, 4]thiazine (XII). To a solution of 0.13 g (1.6 mmole) of isopropylamine and 0.21 g (1.6 mmole) of Et_3N in 40 ml of dry benzene was added over 10-15 min 0.5 g (16 mmole) of (I), and the mixture was stirred for 5 h. The mixture was evaporated to dryness *in vacuo*, and the residue was triturated with ether and kept overnight. The solid was then filtered off and washed with water. The material partly dissolved in the water, and was partly converted into an oil which was extracted with ethyl acetate. The ether layer was evaporated to dryness, and the residue triturated with water and ethyl acetate. The extracts were combined and evaporated, and the residue triturated with ether. The solid was filtered off to give 0.25 g of (XII).

4-Methoxy-6-oxo-7-(4'-carboxyophenyl)amino-5H-pyrimido[4,5-b][1, 4]thiazine (XIII). To a mixture of 0.3 g (2 mmole) of p-aminobenzoic acid and 0.17 g (2 mmole) of pyridine in 30 ml of dry benzene was added portionwise 0.5 g (2 mmole) of (I). The mixture was stirred for 30 min at 18-20°C, then boiled for 2 h. The mixture was evaporated to dryness *in vacuo*, and the residue triturated with water. The solid was filtered off to give 0.4 g of (XII).

2-Chloro-9-oxo-7-(4'-carboxyphenyl)amino-5H-pyrido[2,3-b][1, 4]thiazine (XIV). To a mixture of 0.19 g (2 mmole) of p-aminobenzoic acid and 0.17 g (2 mmole) of pyridine in 30 ml of dry benzene was added portionwise 0.5 g (2 mmole) of (III). The mixture was brought to boiling, and boiled for 2 h. The benzene layer was carefully decanted off, and the oily residue was triturated with water. The resulting solid was filtered off to give 0.5 g of (XIV).

Diethyl N-Methyl-N-[4-methoxy-6-oxo-5H-pyrimido[4,5-b][1, 4]thiazin-7-yl]-p-aminobenzoyl-L-glutamate (XV). A mixture of 0.35 g (1.5 mmole) of (I), 0.5 g (1.5 mmole) of diethyl p-N-methylaminobenzoylglutamate [6] and 0.15 g (1.5 mmole) of Et_3N was stirred in 30 ml of 90% aqueous dioxane for 5 h at 18-20°C. The mixture was evaporated *in vacuo* at ambient temperature until its volume was reduced by approximately a half, then poured into 100 ml of water. The oily material which separated was extracted with ethyl acetate, the extract evaporated *in vacuo*, and the residual oil triturated with alcohol and light petroleum. The solid was filtered off to give 0.65 g of (XV).

Barium N-Methyl-N-[4-methoxy-6-oxo-5H-pyrimido[4,5-b][1, 4]thiazin-7-yl]-p-aminobenzoyl-L-glutamate (XVI). A mixture of 0.6 g (1.1 mmole) of the ester (XV), 0.1 g (2.5 mmole) of NaOH, 2.5 ml of water, and 2.5 ml of methanol was stirred at 18-20°C for 2 h. The solution was acidified with acetic acid and extracted with ethyl acetate. The extract was evaporated *in vacuo*, the residue triturated with dry ether, and the solid filtered off to give a gray powder which deliquesced in air. It was dissolved in 4 ml of water, acidified with acetic acid, and 4 ml of methanol and a solution of 0.55 g (2.2 mmole) of barium acetate added. The solid which separated was filtered off, and dried over P_2O_5 in a desiccator to give 0.4 g of (XVI).

EXPERIMENTAL BIOLOGY

The biological studies were carried out *in vitro* on primary cultures of normal and tumor tissue (chick embryo heart and disseminated rat sarcoma 45). The effects of the compounds were

TABLE 1. Properties of Compounds (I)-(XVI)

Compound	mp, °C (solvent)	Found, %					Empirical formula	Calculated, %					Yield, %
		C	H	N	Cl	S		C	H	N	Cl	S	
I	178—9 (benzene)	36,1	2,7	...	15,0	...	$C_7H_6N_3ClO_2S$	36,3	2,6	18,2	15,3	13,8	85
II	136—8 (cyclohexane)	39,4	3,4	13,9	11,4	10,6	$C_{10}H_{20}N_3ClO_3S$	39,5	3,3	13,8	11,7	10,6	72,3
III	222—4 (toluene)	35,6	1,7	...	30,2	...	$C_6H_4N_2Cl_2OS$	32,3	1,7	12,6	30,1	14,4	85
IV	194—6 (ethanol)	48,5	5,7	23,7	—	...	$C_{12}H_{17}N_5O_2S$	48,4	5,8	23,9	—	...	89
V	163—5 (benzene)	49,4	5,4	19,1	—	...	$C_{15}H_{21}N_5O_4S$	49,1	5,7	19,1	—	...	100
VI	222—5 (benzene)	48,5	5,2		11,6	10,8	$C_{11}H_{15}N_4ClOS$	48,2	5,1		11,8	10,8	92
VII	228—30 (ethanol)	47,1	5,1	19,8	—	11,3	$C_{11}H_{14}N_4O_3S$	46,8	5,0	19,8	—	11,3	96
VIII	162—4 (benzene)	47,3	5,2	15,9	—	9,0	$C_{14}H_{18}N_4O_5S$	47,4	5,1	15,8	—	9,0	87
IX	248—50 (ethanol)	45,9	4,2	14,6	12,2	11,0	$C_{10}H_{12}N_3ClO_2S$	46,2	4,2	14,7	12,4	11,2	98
X	187—90 (benzene)	50,4	4,8	14,5	...	...	$C_{12}H_{14}N_3ClOS$	50,8	4,9	14,8	...	...	82
XI	300 (ethanol + ether)	46,7	3,8	18,4	11,4	10,5	$C_{12}H_{11}N_4ClO_2S$	46,4	3,6	18,2	11,4	10,3	76
XII	170—2 (cyclohexane)	47,5	5,6	22,2	—	...	$C_{10}H_{14}N_4O_2S$	47,2	5,5	22,0	—	...	49
XIII	234—6 (ethanol)	50,6	3,8	16,7	—	...	$C_{14}H_{11}N_4O_4S$	50,5	3,6	16,8	—	...	55
XIV	251—2 (ethanol)	50,0	3,2	...	—	...	$C_{14}H_{10}N_3O_3S$	50,1	3,0	...	—	...	71
XV	149—51 (benzene + ethanol)	54,1	5,4	13,1	—	6,0	$C_{24}H_{26}N_5O_7S$	54,2	5,5	13,1	—	6,0	81
XVI	300 (ethanol + water)	36,3	3,5	10,8	—	...	$C_{20}H_{25}N_5O_{10}BaS$	36,1	3,8	10,5	—	...	53,3

Note. The acid (XVI) was characterized as its Ba salt, which crystallizes with three molecules of water. Water, found 8.1% (calculated 7.8%).

TABLE 2. Cytostatic Activity of the Test Compounds

Compound	Chick embryo heart culture					Sarcoma 45 culture			
	concentration, g/ml								
	10^{-3}	$5\cdot10^{-4}$	$2\cdot10^{-4}$	10^{-4}	10^{-5}	10^{-3}	$5\cdot10^{-4}$	10^{-4}	10^{-5}
IV	—45	—23	...	0	0	—100	—34	0	0
V	—46	—39	...	0	0	—95	—55	0	0
VI	—100	...	...	—56	—30	—100	...	—56	—30
				—60	—40			—60	—40
VII	—90	...	...	—60	0	—80	240	...	0
IX	—80	...	...	—40	0	—80	...	0	0
X	—97	...	...	—52	0	—100	...	—60	0
XI	—60	...	...	—20	0	—100	...	—30	0
XIII	—47	—28	...	0	0	—80	—64	—50	9
XIV	—70	—48	—24	0	0	—100	—86	—60	0
XVI	—65	—45	...	—41	0	—100	...	—65	0

assessed from the extent of suppression of the growth of explants, by measuring the growth zone in the control and experimental groups. Tissue samples (5-8) approximately 0.5 cm × 0.5 cm in size were distributed evenly on the bottom of a Karrel flask in a medium consisting of chicken plasma, medium 199, bovine serum, and embryonic extract. In addition, as soon as a plasma clot was formed, a liquid nutrient medium consisting of 80% medium 199 and 20% of bovine serum with 100 AU/ml of streptomycin and penicillin was added. In the test group of cultures, the test compounds in concentrations of 10^{-3}-10^{-6} g/liter were added to the liquid nutrient medium. The size of the fragments was measured by planimetry using an MBS-1 microscope. Following incubation for 72-96 h at 37°C, the size of the growth zone was measured. The activity of the compound was assessed by its activity coefficient (K_a). The K_a values, expressed as percentages, were calculated using the formula:

$$K_a = \frac{Jt - Jc}{J_c} \cdot 100$$

where J_t is the growth index in the test group, and J_c the growth index in the control group.

Both indices were given by the formula

$$J = \frac{Sz}{S_s},$$

where S_z is the mean area of the growth zone in the group, and S_s the mean size of the samples in the test group.

The effects of the compounds in several concentrations on the embryonic heart and sarcoma 45 are shown in Table 2.

LITERATURE CITED

1. T. S. Safonova and M. P. Nemeryuk, Khim. Geterotsikl. Soedin., No. 5, 714 (1966).
2. T. S. Safonova, N. I. Traven', and N. A. Nersesyan, USSR Inventor's Certificate No. 534,073; Otkrytiya, No. 25, 292 (1982).
3. T. S. Safonova, N. I. Traven', V. A. Chernov, et al., USSR Inventor's Certificate No. 509,048; Otkrytiya, No. 46, 226 (1983).
4. A. S. Sokolova, N. A. Ryabokon', Yu. A. Ershova, et al., Khim.-farm. Zh., No. 9, 49 (1977).
5. L. A. Tyurina, V. A. Semenov, M. P. Nemeryuk, et al., Khim.-farm. Zh., No. 12, 44-50 (1981).
6. D. V. Santi, J. Het. Chem., 4, 475 (1967).