SYNTHESIS AND ANTITUMOR ACTIVITY OF SOME DISUBSTITUTED 5-(3-METHYL-4-ALKOXYBENZYL)PYRIMIDINES

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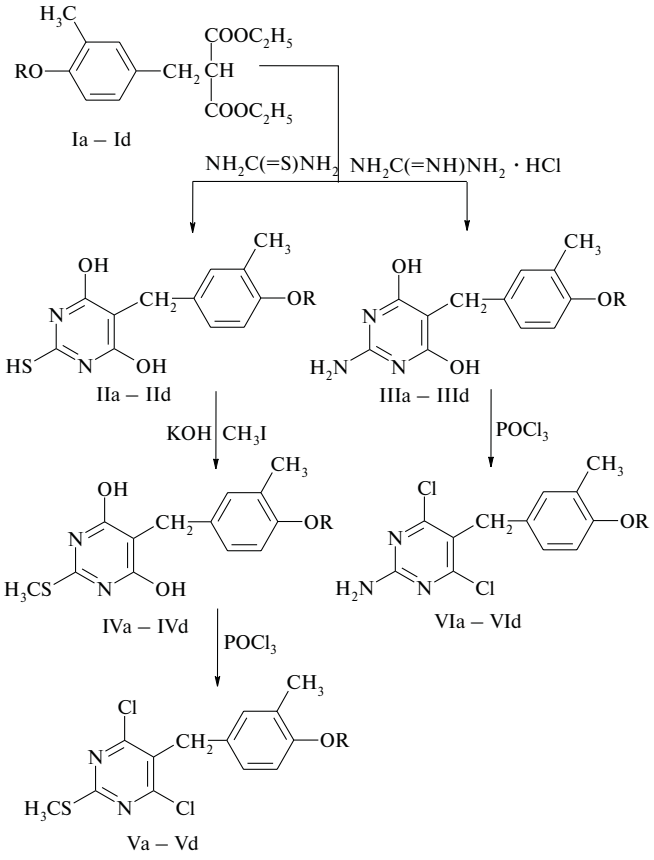
In continuation of the search for new compounds possessing antitumor activity [1], we have synthesized a series of pyrimidines II – VI by the following scheme:



R = Me (a), Et (b), *n*-Pr (c), and *i*–Pr (d).

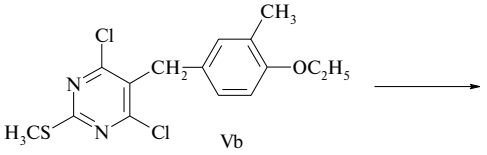
The initial reagents were (3-methyl-4-alkoxybenzyl)malonates (I) obtained by reaction of the corresponding 3-methyl-4-alkoxybenzyl chlorides with malonic ester in the presence of sodium ethylate; the procedure was analogous to that described in [2]. Subsequent cyclization of the substituted malonic esters I with thiourea or guanidine hydrochloride in anhydrous ethanol in the presence of sodium ethylate led to the formation of pyrimidines II or III. Here, an important factor is the amount of sodium ethylate. It was established that 2-mercapto- and 2-amino-4,6-dihydroxypyrimidines II and III can be obtained with high yields only if two or three equivalents of sodium ethylate, respectively, are employed.

Pyrimidines II were converted into methylthio derivatives IV by reaction with methyl iodide in a methanol solution of potassium hydroxide. The purity and identity of compounds II – IV were checked by TLC. The proposed structures were confirmed by data of the IR, ^1H NMR, and mass spectroscopy.

Pyrimidines III and IV were chlorinated by reaction with freshly distilled phosphorus chloroxide (for IV, in the presence of pyridine). The target 4,6-dichloropyrimidines V and VI appear as crystalline substances which, in contrast to the corresponding hydroxy derivatives, are well soluble in many organic solvents.

Finally, we have studied the nucleophilic substitution of diethylaminoethanol for chlorine atoms in 4,6-dichloropyrimidine Vb

The reaction in toluene with a fivefold excess of diethylaminoethanol leads to the formation of a disubstituted product VIII, while the process involving two moles of diethylaminoethanol per mole of Vb yields a monosubstituted pyrimidine VII.



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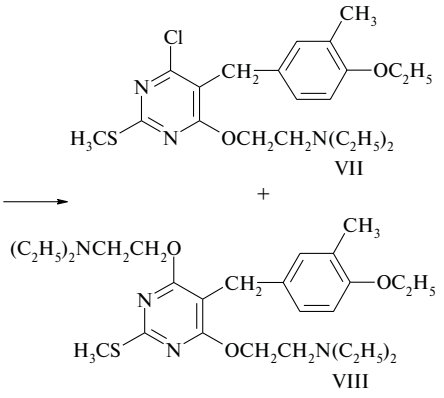

EXPERIMENTAL CHEMICAL PART

The TLC analyses were conducted on Silufol UV-254 plates; the spots were visualized by UV irradiation. The IR spectra were recorded on an UR-20 spectrophotometer (Germany). The ^{1}H NMR spectra were measured on a Mercury 300 spectrometer at a working frequency of 300 MHz, using DMSO-d_6 as the solvent and TMS as the internal standard. The mass spectra were obtained with an MX-1320 spectrometer. The samples were introduced directly into the ionization chamber at a temperature 40 – 50°C below the melting temperature of the corresponding substance and ionized at an electron impact energy of 30 eV. The data of elemental analyses agreed with the results of analytical calculations according to the empirical formulas.

(3-Methyl-4-alkoxybenzyl)malonic acid diethyl esters (Ia – Id). To sodium ethylate prepared from 2.3 g (0.1 g-atom) of metal sodium and 100 ml of anhydrous ethanol were sequentially added 32 g (0.2 mole) of malonic aldehyde and 0.1 mole of the corresponding 3-methyl-4-alkoxybenzyl chloride [3] and the mixture was heated on a water bath with stirring for 10 – 12 h. Then ethanol was distilled off, water added, and the product was extracted with diethyl ether. The ether extracts were dried over calcined sodium sulfate. Then ether was removed and the residues were distilled in vacuum (Table 1).

2-Mercapto-4,6-dihydroxy-5-(3-methyl-4-alkoxybenzyl)pyrimidines (IIa – IId). A mixture of 0.05 mole of (3-methyl-4-alkoxybenzyl)malonate (Ia – Id), 6.08 g (0.08 mole) thiourea, and sodium ethylate prepared from 2.99 g (0.13 g-atom) of metal sodium and 70 ml of anhydrous ethanol was boiled with stirring for 6 – 8 h. Then 120 ml of water was added and the product was extracted with diethyl ether. The aqueous layer was acidified with concentrated HCl until to acid reaction to Congo Red. Upon cooling, the precipitated crystals were filtered, washed with water, and recrystallized from ethanol (Table 2). The parameters of the IR spectra are listed in Table 3.

Compound IIc, ^{1}H NMR spectrum (δ, ppm): 1.05 (t, 3H, $OCH_2CH_2\underline{CH_3}$), 1.75 (m, 2H, $OCH_2\underline{CH_2}CH_3$), 2.10 (s, 3H, CH_3), 3.45 (s, 2H, CH_2), 3.92 (t, 2H, $O\underline{CH_2}CH_2CH_3$), 4.70 (1H, SH) 6.85 (m, 3H, H arom), 11.5 (2H, 2OH).

2-Amino-4,6-dihydroxy-5-(3-methyl-4-alkoxybenzyl) pyrimidines (IIIa – IIId). A mixture of sodium ethylate prepared from 3.45 g (0.15 g-atom) of metal sodium and 100 ml of anhydrous ethanol, 0.05 mole (3-methyl-4-alkoxybenzyl)malonate (Ia – Id), and 4.78 g (0.05 mole) guanidine hydrochloride was boiled with stirring for 10 – 12 h. Then 150 ml of water was added and the mixture was acidified with acetic acid to pH 5 and allowed to stand in a refrigerator for 10 – 12 h. The precipitated crystals were filtered, washed with water, and recrystallized from acetic acid (Table 4). The parameters of the IR spectra are listed in Table 3.

Compound IIIc, ^{1}H NMR spectrum (δ, ppm): 1.00 (t, 3H, $OCH_2CH_2\underline{CH_3}$), 1.70 (q, 2H, $OCH_2\underline{CH_2}CH_3$), 2.05 (s, 3H, CH_3), 3.40 (s, 2H, CH_2), 3.95 (t, 2H, $O\underline{CH_2}CH_2CH_3$), 6.80 (2H, NH_2), 6.90 (m, 3H, H arom), 10.4 (2H, 2OH).

2-Methylthio-4,6-dihydroxy-5-(3-methyl-4-alkoxybenzyl)pyrimidines (IVa – IVd). To 5.6 g (0.1 mole) of potassium hydroxide dissolved on heating in 250 ml of methanol was added 0.1 mole of 2-mercaptopyrimidine (IIa – IId). The mixture was heated on a water bath with stirring for 5 – 10 min and cooled. Then 21.3 g (0.15 mole) of methyl iodide were added and the mixture heated for 15 – 20 min and cooled. Upon cooling, 100 – 150 ml of water were added and the mixture was allowed to stand overnight. The precipitated crystals were filtered, washed with water, and recrystallized from acetic acid (Table 2).

2-Methylthio-4,6-dichloro-5-(3-methyl-4-alkoxybenzyl)pyrimidines (Va – Vd). A mixture of 15.3 g (0.1 mole) of

TABLE 1. Yields and Physicochemical Characteristics of Compounds Ia – Id

Compound	R	Yield, %	B.p., °C/2 Torr	n_D^{20}	d_4^{20}	Empirical formula
Ia	CH_3	60	177 – 178	1.4800	1.0865	$C_{16}H_{22}O_5$
Ib	C_2H_5	70	180 – 182	1.4798	1.0884	$C_{17}H_{24}O_5$
Ic	*n*-C_3H_7	69	178 – 182	1.4787	1.0899	$C_{18}H_{26}O_5$
Id	*i*-C_3H_7	68	178 – 180	1.4780	1.0867	$C_{18}H_{26}O_5$

TABLE 2. Yields and Physicochemical Characteristics of Compounds II and IV

Compound	Yield, %	M.p., °C	R_f	Empirical formula
IIa	90.0	110 – 112	0.49*	$C_{13}H_{14}N_2O_3S$
IIb	88.4	140 – 142	0.51*	$C_{14}H_{16}N_2O_3S$
IIc	72.8	114 – 115	0.50*	$C_{15}H_{18}N_2O_3S$
IId	75.2	104 – 105	0.52*	$C_{15}H_{18}N_2O_3S$
IVa	84.7	210 – 212	0.65**	$C_{14}H_{16}N_2O_3S$
IVb	79.4	232 – 234	0.78**	$C_{15}H_{18}N_2O_3S$
IVc	74.1	267 – 268	0.61**	$C_{16}H_{20}N_2O_3S$
IVd	78.3	259 – 260	0.59**	$C_{16}H_{20}N_2O_3S$

* Petroleum ether – acetone (3 : 2).
** Benzene.

TABLE 3. The IR Spectra of Compounds II – V (ν_{max}, cm^{-1})

Compound	C=C_{arom}	C=N	C–O–C	CH_3	Pyrimidine ring	SH	SCH_3	NH_2, OH	C–Cl
IIa – IId	1600	1655	1186	1420	1520	2620	–	3200, 3320, 3560	–
IIIa – IIId	1950	1680	1210	1420	1530	–	–	3090, 3250, 3390, 3600	–
IVa – IVd	1600	1675	1180	1425	1535	–	1290	3350 – 3500	–
Va – Vc	1580	1700	1180	1440	1540	–	1280	Missing	1720 – 1760

freshly distilled phosphorus chloroxide, 1.6 g pyridine, and 0.01 mole of 2-methylthiopyrimidine (IVa – IVd) was boiled for 8 – 10 h. Then the excess phosphorus chloroxide was distilled off, the residue poured into a glass with ice, and the mixture was allowed to stand overnight. The precipitated crystals were filtered, washed with water, dried, and recrystallized from anhydrous ethanol (Table 5).

2-Amino-4,6-dichloro-5-(3-methyl-4-alkoxybenzyl)pyrimidines (VIa – VId). A mixture of 0.02 mole of 2-amino-4,6-dihydpyrimidines (IIIa – IIId) and 18 ml (0.2 mole) of freshly distilled phosphorus chloroxide was boiled for 10 h. Then the excess phosphorus chloroxide was distilled off, the residue poured into a glass with ice, and the mixture was allowed to stand in a refrigerator for 4 – 5 h. The precipitated crystals were filtered and heated in 25 ml of a 40% aqueous ammonia solution. Upon cooling, the crystals were filtered, washed with water, and recrystallized from acetic acid (Table 5).

2-Methylthio-4-chloro-6-diethylaminoethoxy-5-(3-methyl-4-alkoxybenzyl)pyrimidine (VII). A mixture of 15.3 g (0.05 mole) of 2-methylthio-4,6-dichloropyrimidines Vb and 23.4 g (0.1 mole) of diethylaminoethanol in 30 ml of toluene was boiled for 3 – 4 h. Then 100 ml of water were added and the toluene layer was separated and dried over sodium sulfide. Finally, toluene was removed and the residue was distilled in vacuum; yield of compound VII, 48%; b.p., 250 – 252°C/4 Torr; R_f, 0.65 (petroleum ether); $C_{21}H_{30}ClN_3O_2S$.

2-Methylthio-4,6-di(diethylaminoethoxy)-5-(3-methyl-4-alkoxybenzyl)pyrimidine (VIII). A mixture of 15.3 g (0.05 mole) of 2-methylthio-4,6-dichloropyrimidines Vb and 29.2 g (0.25 mole) of diethylaminoethanol in 30 ml of toluene was boiled for 7 – 8 h and then treated as described above. Compound VIII, 75%; b.p., 265 – 268°C/4 Torr; R_f, 0.60 (petroleum ether – acetone, 3 : 1); $C_{27}H_{44}N_4O_3S$.

TABLE 4. Yields and Physicochemical Characteristics of Compounds IIIa – IIId

Compound	Yield, %	M.p., °C	R_f (methanol)	Empirical formula
IIIa	76.6	> 300	0.69	$C_{13}H_{15}N_3O_3$
IIIb	74.6	> 300	0.66	$C_{14}H_{17}N_3O_3$
IIIc	73.4	289 – 290	0.65	$C_{15}H_{19}N_3O_3$
IIId	75.0	304 – 306	0.60	$C_{15}H_{19}N_3O_3$

EXPERIMENTAL BIOLOGICAL PART

The acute toxicity and antitumor activity of substituted 5-(3-methyl-4-alkoxybenzyl)pyrimidines II, III, and IV were studied by conventional methods [4]. The toxicity was determined by single intraperitoneal injections to white mongrel mice.

The antitumor activity of the synthesized compounds was assessed on mice inoculated with sarcoma 37 by subcutaneous injections of 0.3 ml of a 20% tumor culture suspension in physiological solution. All the compounds tested were injected as suspensions in an 0.5% carboxymethylcellulose solution. The injections were made in a single daily dose of 1/10 LD_{100} over a period of six days beginning with the 4th day after tumor inoculation.

The therapeutic effect was assessed the next day after the last drug injection and evaluated as percentage tumor growth inhibition (TGI). The experimental data were statistically processed by the Student – Fisher method. The differences from untreated control were considered as reliable for $p < 0.05$.

It was established that all the compounds tested possess a low acute toxicity: the absolute lethal dose is 2200 – 2500 mg/kg for compounds III and 1500 – 2000 mg/kg for compounds II and IV. For this reason, the drug doses in the chemotherapy experiments were 150 – 250 mg/kg.

Data on the antitumor activity of 2-mercapto-4,6-dihydroxypyrimidines II showed that the therapeutic doses produced a weak antitumor effect (TGI = 35 – 42%, $p \le 0.05$) while not producing any general toxic effect on the test animals. Among the 2-methylthio-4,6-dihydroxypyrimidine derivatives IV, a reliable protective effect was observed only

TABLE 5. Yields and Physicochemical Characteristics of Compounds V and VI

Compound	Yield, %	M.p., °C	R_f*	Empirical formula
Va	84.5	84 – 85	0.60	$C_{14}H_{14}Cl_2N_2OS$
Vb	81.2	95 – 97	0.62	$C_{15}H_{16}Cl_2N_2OS$
Vc	80.8	87 – 88	0.65	$C_{16}H_{18}Cl_2N_2OS$
VIa	89.6	200 – 202	0.51	$C_{13}H_{13}Cl_2N_3O$
VIb	89.3	188 – 190	0.56	$C_{14}H_{15}Cl_2N_3O$
VIc	85.4	159 – 160	0.53	$C_{15}H_{17}Cl_2N_3O$

* Diethyl ether – petroleum ether (1 : 4).

for compounds with ethoxy (IVb) and propoxy (IVc) radicals (TGI = 40 – 43%, $p < 0.05$). In contrast to the mercapto and methylthio derivatives, aminopyrimidines III possessed a somewhat more pronounced antitumor properties. For example, the ethoxy (IIb) and propoxy (IIIc) derivatives showed TGI = 55 and 62%, respectively ($p < 0.05$).

Thus, some of the newly synthesized 5-substituted 5-(3-methyl-4-alkoxybenzyl)pyrimidines possess a significant antitumor activity in combination with low toxicity.

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