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PYRIMIDINE DERIVATIVES.

XLIX. SOME DERIVATIVES OF THE 2-SUBSTITUTED PHENOXYMETHYL-4-HYDROXY-6-METHYLPYRIMIDINES AND THE STUDY OF THEIR ANTITUMOR ACTIVITY

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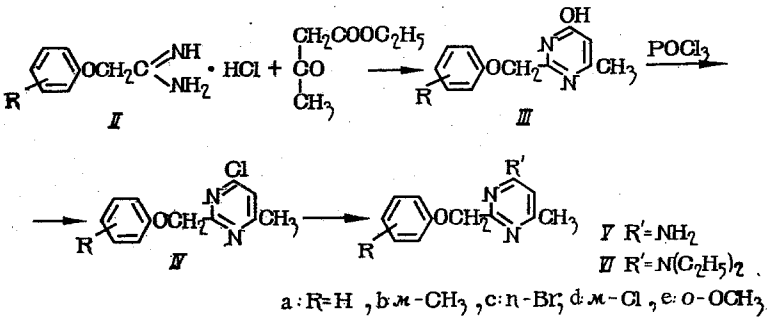
We have previously synthesized a series of 4-substituted pyrimidines with various functional groups in positions 2,5, and 6; compounds of interest as antimetabolites in nucleic metabolism [1-3].

Continuing the investigation in the elucidation of the connection between the structure of pyrimidines and their antitumor activity, 4-hydroxy, chloro-, and aminopyrimidines containing a phenoxymethyl group in position 2 (III-VI) are described, and studies on these compounds are presented in this work.

Compounds III-VI were prepared according to the following sequence:



The 4-hydroxypyrimidines III compounds were prepared by the cyclization of amidine hydrochlorides of the substituted phenoxyacetic acids with acetoacetic ester in the presence of

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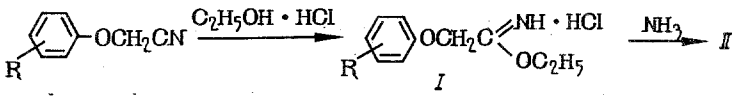
sodium ethylate. Starting amidine hydrochlorides II were prepared from the corresponding nitriles [4] with the isolation of the intermediate hydrochlorides of the phenoxyacetic acid iminoesters I.

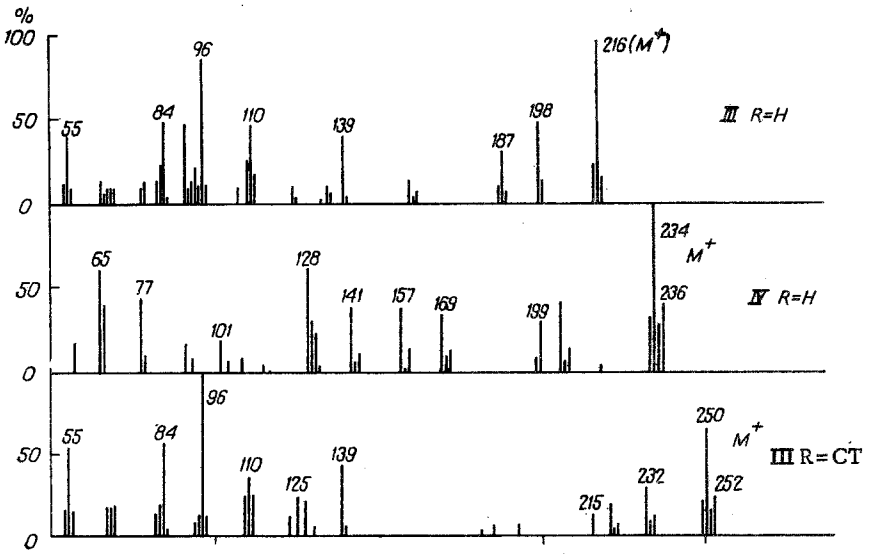


Substitution of the hydroxyl group with chlorine in hydroxypyrimidines III was carried out by heating the latter with an excess of phosphorus oxychloride. The use of pyridine or dimethylaniline did not increase the yield over 50-55%. The best results in the amination of chloropyrimidines IV were obtained on heating IV in ethanolic ammonia or diethylamine in an autoclave at 150°C for 5-6 h.

The 4-amino- and diethylaminopyrimidines were converted into the corresponding water-soluble hydrochlorides by the action of the ethereal solution of hydrogen chloride, for the study of their biological properties.

Purity of the 4-substituted pyrimidines was checked by thin-layer chromatography, and their structure was confirmed by elemental analysis, IR, and mass spectral data.

IR spectrum of III showed a strong absorption around 1635 cm^{-1} (C=O) and 3130 cm^{-1} (amide NH) due to the lactame structure of 4-hydroxypyrimidines III. Weak and board absorption around 3300 cm^{-1} (hydroxy-group stretching vibrations) and 1580 cm^{-1} (pyrimidine aromatic nucleus vibrations) also indicated the presence of the hydroxy-form.



Mass spectra of compounds III, IV (R=H, Cl).

Mass spectra of IIIa,b (R=H, Cl) and Va (R=H), which are presented, allowed an easy identification of their structures by using 6-8 peaks belonging to the characteristic ions. Basic fragmentation patterns were confirmed by the corresponding peaks of the metastable ions.

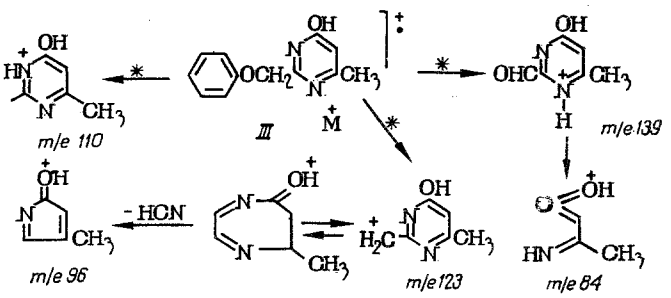


TABLE 1. Summary Data on the Toxicity and Antitumor Activity of Compounds IIIa-e, Va-e, and VIa,b

Compound	Acute toxicity to mice			Antitumor activity					
				rats	mice				
					% inhibition				
	LD_{100}	LD_{50}	maximal tolerated dose	dose, mg/kg	sarcoma 45	carcinosarcoma Walker 256	dose, mg/kg	sarcoma 180, % inhibition	Ehrlich ascites tumor, half-life increase, %
	mg/kg								
IIIa	510	390 (325—468)	305	50	0	0	100	+	0
IIIb	900	750 (645—875)	460	80	±	0	150	+	
IIIc	750	575 (515—643)	500	50	0	±	75	0	0
IIId	1500	1250 (1087—1437)	1050	100	0	0	250	+	0
IIIд	765	600 (517—690)	460	50	0	±	75	+	0
Va		600 (517—696)	415	70	±	+	100	+	0
VIa	520	400 (342—468)	265	50	+	+	100	+	47,4
Vb	625	450 (381—531)	310	60	+	+	100	+	0
Vc	630	400 (333—480)	265	60	+	0	100	±	51
Vd	625	(435—575)	415	60	+	±	100	+	0
VId	410	300 (248—363)	210	40	+	+	75	+	42,8
Ve	630	450 (375—540)	305	60	0	±	100	+	0

Note. 0 = no effect; ± = inhibition of the tumor growth of up to 30%; + = inhibition of 30-59%.

TABLE 2. Iminoesters and Amidines of the Substituted Phenoxyacetic Acids

Compound	Yield, %	mp, °C	Found, %		Molecular formula	Calculated, %	
			N	Cl		N	Cl
Ia	70,4	114—5	6,05	16,24	$C_{10}H_{13}NO_2 \cdot HCl$	6,49	16,43
Ib	68,3	111—2	6,58	15,87	$C_{11}H_{15}NO_2 \cdot HCl$	6,09	15,43
Ic	72,5	95—6	5,06	11,92	$C_{10}H_{12}NO_2Br \cdot HCl$	4,75	12,03
Id	71,7	84—5	5,22	13,91	$C_{10}H_{12}ClNO_2 \cdot HCl$	5,60	14,17
Ie	65,8	74—5	5,20	13,99	$C_{11}H_{15}NO_3 \cdot HCl$	5,70	14,43
IIa	76,7	127—8	15,31	18,60	$C_8H_{10}NO \cdot HCl$	15,01	18,99
IIb	69,0	182—3	14,20	17,96	$C_9H_{12}NOO \cdot HCl$	13,96	17,66
IIc	65,0	108—9	10,35	13,11	$C_8H_9BN_2O \cdot HCl$	10,51	13,30
IId	60,3	100—1	13,31	16,05	$C_8H_{12}N_2O_2 \cdot HCl$	12,92	16,36

Note. Hydrochlorides of iminoesters Ia-e melt with decomposition. Ref. [2], mp Ia (R-H) 111-113°C; Ib ($R-CH_3$) 108.5-111°C; mp IIa 127.5-128.5°C; IIb 179-180°C. Hydrochlorides of iminoesters Ia-e melt with decomposition.

EXPERIMENTAL

Pharmacological

Toxicity and antitumor activities of compounds III, V, and VI were determined according to V. A. Chernov [5] and the results are presented in Table 1. Toxicity was studied on white mongrel mice weighing 18-20 g, using a single intraperitoneal administration, while the chemotherapeutic experiments were carried out on rats and mice with implanted tumors (sarcoma 45, carcinosarcoma Walker 256, sarcoma 180, and Ehrlich ascites tumor).

LD_{100} for 4-hydroxypyrimidines III was 500-1500 mg/kg, whereby the substitution of the phenyl radical atom in various positions decreased somewhat the toxicity of the compounds (Table 1). LD_{100} for hydrochlorides V and VI was 760 mg/kg. It should be pointed out that compounds containing a diethylamine group were somewhat more toxic.

The study of the antitumor properties showed that derivatives III were inactive against the types used, with the exception of IIIa, b, and d, which inhibited the growth of sarcoma 180 by 30-59%. Compounds V and VI showed a moderate inhibiting activity with sarcromas 45 and 180, and carcinosarcoma Walker 256. Some of the compounds studied inhibited the growth of Ehrlich ascites tumor and extended the life of mice by 40-50%.

Chemical

Thin-layer chromatography was done on Silufol UV-254 plates with UV visualization. IR spectra were taken on an UR-20 spectrophotometer using a mineral oil mull. Mass spectra were recorded with a MX-1303 instrument using a direct injection of the sample into the ion source at a temperature 20-30°C lower than the mp of the investigated compound. LD_{50} was determined according to Lichfield-Wilcoxon [6].

Hydrochlorides of the Ethyl Iminoesters of the Substituted Phenoxyacetic Acids (I). A mixture of substituted phenoxyacetonitrile (0.42 moles) and absolute ethanol (20 g, 0.44 moles) was cooled to 0-5°C and saturated with dry hydrogen chloride (16 g, 0.44 moles). Absolute ether was then added, the crystalline precipitate collected, thoroughly washed with absolute ether, and dried in a vacuum desiccator over phosphorus pentoxide (Table 2).

Amidine Hydrochlorides of the Substituted Phenoxyacetic Acids (II). Absolute ethyl alcohol containing 0.13 moles of ammonia (50 ml) was added gradually at 0-5°C to I (0.1 mole). After 30 min the temperature of the reaction mixture was raised to room temperature and stirring was continued for 2 days. Crystalline ammonium chloride was then filtered, the ethanol removed, and the residue crystallized from absolute ether. Crystals were collected, washed with absolute ether, and dried in a vacuum desiccator over phosphorus pentoxide (Table 2).

2-Substituted Phenoxymethyl-4-hydroxy-6-methylpyrimidines (III). To a cooled solution of sodium ethylate, prepared from sodium (4.6 g, 0.2 moles) and absolute ethanol (100 ml), was added acetoacetic ester (0.1 moles). The reaction mixture was heated on a water bath for 3-4 h. After removal of the solvent, the residue was dissolved in water (50 ml), cooled, and acidified with glacial acetic acid to pH 6.0-7.0. Crystalline precipitate was collected and washed with ice water. Crude product was dissolved in 10% sodium hydroxide solution, filtered, and the filtrate acidified with acetic. Crystalline precipitate was collected and dried in the air (Table 3).

2-Substituted Phenoxymethyl-4-chloro-6-methylpyrimidines (IV). A mixture of III (0.02 moles) and phosphorus oxychloride (30 g, 0.2 moles) was heated on a water bath for 4-5 h. After removal of the excess of phosphorus oxychloride, the residue was poured on finely ground ice. In the case of IV (R=Cl, CH_3, OCH_3) yellow crystals precipitated, while with R=H, Br in oil was extracted with ether. The ether extracts were washed with 10% sodium hydroxide solution, followed with water, and dried over anhydrous sodium sulfate. After removal of the ether, the residue was distilled under reduced pressure. Thin-layer chromatography was carried out with a system of ether–petroleum ether (2:1) (Table 3).

2-Substituted Phenoxymethyl-4-amino-6-methylpyrimidines (V). A mixture of IV (0.01 mole) and a solution of ammonia (0.84 g, 0.04 mole) in ethanol (30 ml) was heated in an autoclave at 150°-160°C for 5-6 h. The ethanol was removed and water added to the residue, followed by ether extraction. The ether extracts were washed with a 5% sodium hydroxide solution, followed by water, and dried over anhydrous sodium sulfate. After removal of the ether, the residue was recrystallized from water. Thin-layer chromatography was carried out with a system of methanol:chloroform (1:1) for IV (R=H) and with a system of ethanol:chloroform (2:1) for R=CH_3, Br, Cl, OCH_3 (Table 3).

2-Substituted Phenoxymethyl-4-diethylamino-6-methylpyrimidines (V). These were prepared analogously to the components described above from IV (0.01 mole) and diethylamine (0.03 moles) in ethanol (30 ml).

VI (R=H), 1.8 g, 68.5%, bp 180-182°C (1 mm Hg). Found, %: C 70.51; H 7.46; N 15.17. $C_{16}H_{21}N_3O$. Calculated, %: C 70.81; H 7.80, N 15.48. Hydrochloride, mp 96-97°C.

TABLE 3. 2-Phenoxymethyl-4-substituted Pyrimidines III-V

Compound	Yield, %	bp, °C*	mp, °C	R_f	Found, %				Molecular formula	Calculated, %				Hydrochlorides, mp °C
					C	H	N	Cl		C	H	N	Cl	
IIIa	80,5	—	155—6		65,92	5,53	12,74	—	$C_{12}H_{12}N_2O_2$	66,65	5,59	12,95	—	—
IIIb	73,6	—	118—20		67,64	6,41	12,42	—	$C_{13}H_{14}N_2O_2$	67,81	6,13	12,17	—	—
IIIc	87,5	—	185—6		43,29	3,36	7,85	—	$C_{12}H_{11}BrN_2O_2$	43,65	3,02	7,33	—	—
IIId	78,4	—	187—8		57,25	4,45	11,21	—	$C_{12}H_{11}ClN_2O_2$	57,49	4,42	11,17	—	—
IIIe	72,6	—	138—9		62,92	5,69	11,90	—	$C_{13}H_{14}N_2O_3$	63,40	5,73	11,83	—	—
IVa	56,5	160—65	—	0,60	—	—	11,76	14,83	$C_{12}H_{11}ClN_2O$	—	—	11,94	15,11	—
IVb	52,3	170—73	38—9	0,52	—	—	11,00	14,76	$C_{13}H_{13}ClN_2O$	—	—	11,25	14,26	—
IVc	48,6	175—76	36—7	0,58	—	—	9,15	11,22	$C_{12}H_{10}BrClN_2O$	—	—	9,23	11,68	—
IVd	50,3	166—67	83—4	0,54	—	—	16,26	26,57	$C_{12}H_{10}Cl_2N_2O$	—	—	16,56	26,36	—
IVe	57,4	—	53—4	0,50	—	—	10,95	12,97	$C_{13}H_{13}ClN_2O_2$	—	—	10,58	13,39	—
Va	75,4	250—55	120—2	0,65	66,53	6,45	15,68	—	$C_{12}H_{13}N_3O$	66,96	6,09	15,92	—	116—7
Vb	69,5	225—30	88—9	0,59	68—17	6,71	18,04	—	$C_{13}H_{15}N_3O$	68,07	6,59	18,32	—	161—2
Vc	60,2	—	154—5	0,63	48,70	4,30	14,68	—	$C_{12}H_{12}BrN_3O$	48,99	4,11	14,28	—	193—4
Vd	75,0	—	124—5	0,75	57,50	4,64	17,25	13,81	$C_{12}H_{12}ClN_3O$	57,72	4,84	16,83	14,20	184—5
Ve	73,3	—	158—9	0,54	64,01	5,91	17,18	—	$C_{13}H_{15}N_3O_2$	63,66	6,16	17,13	—	201—2

*Residual pressure 1 mm Hg.

VI (R-m-Cl)R-m-Cl, 1.9 g, 70.0%, bp 190-195°C (1 mm Hg). Found, %: C 63.07; H 6.23; N 13.45; Cl 11.78. $C_{16}H_{20}ClN_3O$. Calculated, %: C 62.84; H 6.59; N 13.74; Cl 11.59. Hydrochloride, mp 149 - 150°C.

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