73. Ph. Tcherdakoff, Pharmatherapeutica, 3, 342-348 (1983).
74. M. Thibonnier, M. D. Lardoux, and P. Corvol, Br. J. Clin. Pharmacol., 9, 561-567 (1980).
75. K. Tomioka, T. Yamada, and T. Takenaka, Arch. Int. Pharmacodyn., 256, 97-107 (1982).
76. Y. Uchida, M. Nakamura, S. Shimizu, et al., Arch. Int. Pharmacodyn., 262, 132-139 (1983).
77. E. M. Vaughan Williams, J. S. Millar, and T. J. Campbell, Cardiovasc. Res., 16, 233-239 (1982).
78. A. J. M. Verberne and M. J. Rand, Eur. J. Pharmacol., 108, 193-196 (1985).
79. H. J. Waal-Manning and F. O. Simpson, Br. J. Clin. Pharmacol., 13, No. 1, Suppl. 65S-73S (1982).
80. J. J. Walker, I. A. Greer, M. McLaren, et al., Br. J. Obstet. Gynaec., 90, 1094-1098 (1983).
81. J. K. Woodward and H. C. Cheng, J. Pharm. Pharmacol., 34, 193-195 (1982).
82. H. Zschedrich, W. Neuroh, J. Lutt, et al., Klin. Wochenschr., 61, 661-667 (1983).

SYNTHESIS AND BIOLOGICAL ACTIVITY OF N'-ALKOXYBENZENESULFONYL-5-HALOURACILS

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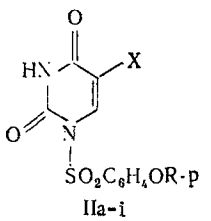
The introduction into 5-fluorouracil (Ia) of an alkoxybenzyl substituent gives N-substituted uracils possessing antitumor activity equal to that of (Ia), but of lower toxicity [2].

Continuing studies of the modification of the structure of (Ia), we here report the synthesis of some novel N'-4-alkoxybenzenesulfonyl-5-fluoro (and bromo) uracils (IIa-i).



IIa-i

IIa, f: R = Me; IIb, g: R = Et; IIc, h: R = Pr; IId, i: R = Bu; IIe: R = Bu-*i*; IIa-e: X = F; IIf-i: X = Br.

The rationale for the synthesis of (IIa-i) was the observation that the introduction of the sulfonyl group into (Ia) reduces its toxicity [6, 7].

The starting materials employed were (Ia) and 5-bromouracil (Ib) [10], together with 4-alkoxybenzenesulfonyl chlorides, obtained by chlorosulfonation of alkoxybenzenes in a large excess of freshly-distilled chlorosulfonic acid [9].

An examination of the reactions of (Ia) and (Ib) with 4-alkoxybenzenesulfonyl chlorides in DMSO in the presence of LiH or aqueous-alcoholic KOH showed that low yields of N'-substituted products were obtained. The best results were obtained by heating a mixture of (Ia) or (Ib) with the 4-alkoxybenzenesulfonyl chloride and anhydrous potassium carbonate in DMF at 70-80°C.

The position of the benzenesulfonyl grouping was established by mass spectrometry. In addition to the molecular peak at 300, ions were found having m/z 236 ($M - SO_2$), 193 (236 − HNCO). The presence of the latter ion, and the absence of an ion with m/z 213, showed that the 4-methoxybenzenesulfonyl group is located in the 1-position of (Ia).

The purities and homogeneity of (IIa-i) were checked by chromatography.

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TABLE 1. Properties of N'-4-Alkoxybenzenesulfonyl-5-bromo (or fluoro)uracils (IIa-i)

Compound	Yield, %	mp, °C	R_f	Found, %		Empirical formula	Calculated, %	
				N	S		N	S
IIa	80,0	207—8	0,56	9,69	10,24	$C_{11}H_9FN_2SO_5$	9,33	10,66
IIb	70,0	185—6	0,63	9,03	10,44	$C_{12}H_{11}FN_2SO_5$	8,91	10,20
IIc	85,3	150—51	0,69	8,32	9,54	$C_{13}H_{13}FN_2SO_5$	8,53	9,76
IId	54,7	151—2	0,71	8,32	9,60	$C_{14}H_{15}FN_2SO_5$	8,18	9,36
IIe	68,0	184—5	0,73	8,42	9,16	$C_{14}H_{15}FN_2SO_5$	8,18	9,36
IIf	40,9	205—6	0,61	8,08	8,48	$C_{11}H_9BN_2SO_5$	7,76	8,86
IIg	61,4	180—81	0,67	7,06	9,55	$C_{12}H_{11}BN_2SO_5$	7,19	8,53
IIh	43,0	164—5	0,68	7,08	8,61	$C_{13}H_{13}BN_2SO_5$	7,19	8,22
IIi	45,1	171—2	0,67	6,73	7,96	$C_{14}H_{15}BN_2SO_5$	6,94	7,94

TABLE 2. Chemotherapeutic Activity of N'-4-Alkoxybenzenesulfonyl-5-bromouracils

Compound	Microorganism (infective dose)	Strain	Overall lifespan of animals	
			abs.	%
IIf	Staphylococci (800 million microbial cells)	4-0	10/50	20
IIg			20/50	40
IIh			20/50	40
IIi			20/50	40
Norsulfazole			30/50	60
Control	The same	Smith	0/100	0
IIf			20/50	40
IIg			30/50	60
IIh			20/50	40
IIi			. . .	. . .
Norsulfazole			40/50	80
Control	Dysentery bacilli Flexner (200 million microbial cells)	6858	10/50	20
IIf			10/50	20
IIg			10/50	20
IIh			20/50	40
IIi			10/50	20
Norsulfazole			10/50	20
Control			0/50	0

Note. The numerator denotes the number of mouse-days in the group, and the denominator the maximum possible number of mouse-days for an observation period of ten days.

TABLE 3. Antitumor Activity of (IIa-e) against Sarcoma 180

Compound	Dose, mg/kg	Inhibition of tumor growth, %	P
IIa	100	31	0,05
IIb	75	38	0,05
IIc	75	42	0,05
IId	100	52	<0,05
IIe	100	52	<0,05

EXPERIMENTAL (CHEMICAL PART)

Mass spectra were obtained on an MX-1303 instrument, with direct introduction of the sample into the ionization zone, the ionizing electron energy being 30 eV and the temperature 30-40°C below the melting point. Chromatography was carried out on Silufol UV-254 plates in the system dry ether—light petroleum (1:0.06 for (IIa-e), 5:1 for (IIf-i)), the spots being visualized with a UI-1 ultrachemiscope.

N'-4-Alkoxybenzenesulfonyl-5-halouracils (IIa-i). A mixture of 0.04 mole of the 5-halouracil, 2.8 g (0.02 mole) of dry potassium carbonate, and 150 ml of DMF was heated on the water bath at 70-80°C for 1 h. After cooling, 0.02 mole of the 4-alkoxybenzenesulfonyl chloride in 40 ml of DMF was added, and heating continued for 5-6 h. The mixture was then cooled, and poured into 400 ml of cold water, acidified with conc. HCl to pH 4.0-5.0, and the crystals which separated were filtered off, washed with water, and recrystallized from ethanol (Table 1).

EXPEIRMENTAL (BIOLOGICAL PART)

The antibacterial activity of the test compounds was assessed in model generalized staphylococcal and dysenteric infection in white mice, induced by intraperitoneal administration of a test culture [4]. Two strains of staphylococci (Smith and 4-0) and the Flexner dysentery bacillus strain 6858 were used. The drugs were administered in a single dose at the same time as infection. The activity of the compounds was calculated from the increased lifespan of the mice as a percentage of the maximum possible for the group, observations being continued for ten days. Norsulfazole was used in parallel experiments.

Antitumor activity was examined by a standard method [8] in white rats and mice with transplanted tumors (sarcomas 45 and 180, Walker's carcinoma, and Erlich's ascitic carcinoma).

The mutagenic and antimutagenic effects of the compounds were examined in the biochemical strains: *Escherichia coli* P-678 auxotrophic in threonine, and *Actinomyces rimosus* 222, auxotrophic in lysine. The studies were carried out by the dose–effect method [5]. Mutagenic activity was assessed from the frequency of occurrence of inverse mutations, at loci responsible for the synthesis of threonine and lysine. Antimutagenic activity was assessed from the effects on spontaneous mutations and on UV-induced mutations. UV irradiation was carried out as described in [3]. The protectants 2-mercaptoethylamine and cystamine were used as positive controls. The results obtained were treated statistically [1].

Antimicrobial and antitumor activity, together with the acute toxicities of the drugs, were examined in mongrel mice and rats of both sexes weighing 16-20 and 90-110 g, respectively. 570 mice and 180 rats were used. The absolute lethal dose (LD_{100}) was determined in mice following a single intraperitoneal dose, the values for (IIa-e) being, depending on the alkoxyradical, 750-1250 mg/kg, and for (IIf-i) greater than 2500 mg/kg. The maximum tolerated dose of fluorouracils (IIa-e) was 2000-2500 mg/kg. In a dose of 2500 mg/kg, the bromo-compounds (IIf-i) did not give rise to any perceptible changes in the behavior or condition of the animals. Higher doses were not tested.

Examination of the antibacterial properties of (IIa-e) showed them, in tests on staphylococcal infection, to be without effect on the lifespan of the animals infected with the Smith and 4-0 strains, in doses of 1000 and 1500 mg/kg, as compared with the control (untreated) animals. In a dose of 1500 mg/kg, the bromouracils significantly extended the lifespan of animals infected with the Smith strain, generally by 40% ($0.01 < P < 0.001$), and with the 4-0 strain by 20-40% ($P < 0.001$). Norsulfazole increased the lifespan of the animals by 80 and 60%, respectively ($P < 0.001$). In dysenteric infection (IIf-i), like norsulfazole, increased the lifespan of the animals by 20% ($P < 0.01$) (Table 2).

A study of antitumor activity showed that in doses of 1/10 and 1/20 of the LD_{100} the fluoro-compounds significantly inhibited the growth of sarcoma 180, by 31-52%. The most active compound was (IIe), which has an isobutyloxyradical, and which also had an inhibitory effect (by 60%) in sarcoma 45. The bromo-compounds (IIf-i), at the same doses, showed no antiblastic activity against the strains employed (Table 3).

Studies of the mutagenic effects of (IIa-i) showed that at the same decimolar concentrations and time of treatment of the test cultures they showed very low mutagenic activity (the results were statistically nonsignificant).

The antimutagenic activity of (IIa-i) was examined at much lower molar concentrations and time of treatment of the same test cultures. Two compounds (IIa and IIf) were found to possess antimutagenic activity. The results of these studies are given in Table 4. At high cell (spore) viability, compound (IIa) reduced the number of mutations as compared with those arising spontaneously in the controls: in *E. coli* by 25%, and in *Actinomyces* by 48%. Compound (IIf) had a similar effect only in *Actinomyces*, reducing the number of mutations by 35%. Compound (IIa) was also active in mutations induced by UV irradiation. At high *Actinomyces* spore viability, it reduced the number of mutations by 40%. The control protectants in the same test systems had approximately the same protectant activity as the test compounds.

TABLE 4. Effects of Uracils (IIa) and (IIf) and of Control Protectants on Spontaneous and UV-induced Mutations in Test Cultures

Compound	Dose		Effect on spontaneous mutations						Effect on UV mutations		
			E. coli P-678 thr⁻			Act. rimosus 222 lys⁻			Act. rimosus 222 lys⁻		
	mmole	min	survival, %	incidence of revertants per 10^6 surviving cells		survival, %	incidence of revertants per 10^5 surviving spores		survival, %	incidence of revertants per 10^5 surviving spores	
				abs	% of controls		abs	% of controls		abs	% of controls
IIa	10	10	86	4,5±0,6	75	120	2,1±0,3	52	160	2,4±0,3	60
IIf	10	10	90	6±0,75	100	92	2,6±0,25	65	85	4,4±0,5	110
2-Mercaptoethylamine	100	10	140	3,27±0,4	54,5	96	2,1±0,25	52,5	175	2,34±0,2	58,4
Cystamine	25	10	97	5,58±0,65	93	106	2,13±0,2	53	127	3±0,35	75
Spontaneous mutations (control)			100	6±0,7	100	100	4±0,5	100	...	...	...
UV mutations (control)			...	...	...	...	...	...	100	4±0,35	100

A study of the dependence of the biological activity of (IIa-i) on structure shows that the introduction of a bromine atom into the 5-position of uracil (IIa-f) gives rise to antibacterial activity, whereas antimutagenic and radioprotectant properties, together with antiblastic activity, appear when the bromine is replaced by fluorine. It has also been found that lengthening the alkoxy radical in these compounds results in a loss of antimutagenic properties or enhancement of antibacterial activity.

Some N'-alkoxybenzenesulfonyl-5-fluoro(bromo)uracils have thus been found which possess antibacterial, antiblastic and antimutagenic activity, providing justification for continued studies in this area.

LITERATURE CITED

1. M. L. Belen'kii, Fundamentals of the Quantitative Measurement of Pharmacological Activity [in Russian], 2nd ed., Leningrad (1963).
2. R. G. Melik-Ogandzhanyan, R. G. Mirzoyan, V. E. Khachatryan, et al., Khim.-farm. Zh., No. 5, 38-40 (1978).
3. G. Miller, Experiments in Molecular Genetics [Russian translation], Moscow (1976), pp. 112-115.
4. E. N. Padeiskaya, Methods of Experimental Chemotherapy [in Russian], Moscow (1971), pp. 109-109, 143-143.
5. G. M. Paronikyan, L. G. Akopyan, and M. G. Oganesyan, Genetika, 7, No. 4, 113-117 (1971).
6. US Patent No. 3,971,784; Izobret. za rubezhom., No. 24, 43 (1976).
7. Japanese Patent No. 53-24951; ibid., No. 4, 182 (1970).
8. V. A. Chernov, Methods of Experimental Chemotherapy [in Russian], Moscow (1971), pp. 6, 357.
9. M. S. Morgan and L. H. Cretcher, J. Am. Chem. Soc., 70, 375-380 (1948).
10. S. J. Wang, J. Org. Chem., 34, 11-13 (1959).

SYNTHESIS OF AN INDOLE ANALOG OF FOLIC ACID

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Of the numerous studies devoted to modifications of the structure of folic acid, only a few have been concerned with replacement of the p-aminobenzoic acid (PABA) moiety. Pyridine, piperidine, and thiazole analogs of folic acid have been synthesized [10-12].

In view of the similarity of the electronic and spatial structures of PABA to those of 5-aminoindole-2-carboxylic acid, we have synthesized an indole analog of folic acid, namely dimethyl N-[5-(2'-amino-4'-oxo-6'-pteridinyl)methylaminoindol-2-yl]glutamate (XII). This was obtained by the reductive condensation of 6-pterinaldehyde (X) with dimethyl 5-aminoindol-2-ylglutamate (VII).

The starting material for the synthesis of the dimethyl ester (VII) was 5-nitroindole-2-carboxylic acid (I) [3]. Acylation of glutamic acid with 5-nitroindole-2-carbonyl chloride (II) [5] in aqueous sodium bicarbonate or sodium hydroxide did not give the desired results, the acid chloride (II) being hydrolyzed to the acid (I) and the glutamic acid remaining unchanged. Acylation in other solvents (DMF, pyridine, methylene chloride) was also unsuccessful. On the other hand, dibenzyl, dimethyl, and diethyl glutamates reacted smoothly with the acid chloride (II) in ethyl acetate in the presence of triethylamine to give the corresponding esters

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