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PYRIMIDINES.

LIX. SYNTHESIS AND BIOLOGICAL PROPERTIES OF N-SUBSTITUTED DIHYDROURACILS AND DIHYDROTHIOURACILS

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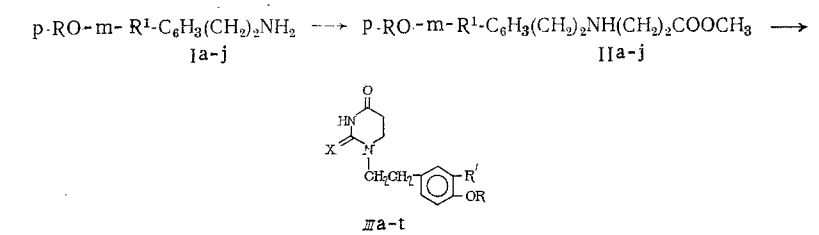
N-substituted pyrimidines with p-alkoxybenzyl groups have been described in earlier reports [1-3]. In continuation of these studies, in order to elucidate the relationship of biological properties to structure, we have synthesized some new dihydrouracils and dihydrothiouracils (IIIa-t), containing N-phenethyl groups, from phenethylamines (Ia-j).



IIIa-t

Ia, g, IIa, g: R = CH_3; Ib, h, IIb, h R = C_2H_5; Ic, i, IIc, i: R = C_3H_7; Id, l, IId, l: R-iso-C_3H_7; Ie, IIe: R = C_4H_9; If, IIf: R-iso-C_3H_7; Ia-f, IIa, f R^1 = H; Ig, j, IIg-j R^1 = CH_3; IIIa: R = CH_3, R^1 = H, X = O; IIIb R = C_2H_5; R^1 = H, X = O; IIIc. R = C_3H_7, R^1 = H, X = O; IIId: R-iso-C_3H_7, R^1 = H, X = O; IIIe. R = C_4H_9, R^1 = H, X = O; IIIf: R-iso-C_4H_9, R^1 = H, X = O; IIIg: R = R^1 = CH_3, X = O; IIIh R = C_2H_5, R^1 = CH_3, X = O; IIIi: R = C_3H_7, R^1 = CH_3, X = O; IIIj: R-iso-C_3H_7, R^1 = CH_3, X = O; IIIk: R = CH_3, R^1 = H, X = S; IIIl: R = C_2H_5, R^1 = H, X = S; IIIm: R = C_3H_7, R^1 = H, X = S; IIIn: R = iso-C_3H_7, R = H, X = S; IIIl: R = C_4H_9, R^1 = H, X = O; IIIp: R = iso-C_4H_9, R^1 = H, X = S; IIIo R = R^1 = CH_3, X = S; IIIr: R = C_2H_5, R^1 = CH_3, X = S; IIIs: R = C_3H_7, R^1 = CH_3, X = S; IIIq: R = iso-C_3H_7, R^1 = CH_3, X = S.

The starting materials (Ia-j) were obtained by reducing p-alkoxy- and m-methyl-p-alkoxybenzyl cyanides over a standard industrial catalyst consisting of nickel on chromium oxide, as described in the literature [4, 5]. Reaction with methyl acrylate in absolute methanol at ambient temperature converted the amines (Ia-j) into the methyl β-(phenethylamino)propionates (IIa-j). Reaction of the latter with urea or ammonium thiocyanate in an acid medium gave the dihydrouracils and dihydrothiouracils (IIIa-t).

The purities of the compounds (IIIa-t) were checked by TLC, and their structures established by mass spectrometry. The mass spectra of (IIIi) and (IIIq) showed peaks for the molecular ions, together with a number of fragment peaks (290, 206, 177, 164, 134, 107, 292, 208, 162, 134, 116, 107), the derivation of which confirmed their structures.

EXPERIMENTAL CHEMISTRY

Mass spectra were obtained on an MX-1303 instrument with direct introduction of the sample into the ionization region at a temperature 40-50°C below the melting point, ionizing electron energy 30 eV. Chromatography

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TABLE 1. Methyl β-(Phenethylamino)propionates (IIa-j)

Compound	Yield, %	bp, °C (mm)	d_4^{20}	n_D^{20}	Found, %			Empirical formula	Calculated, %		
					C	H	N		C	H	N
IIa	76,7	172—173 (2)	1,0788	1,5028	65,62	8,52	6,04	$C_{13}H_{19}NO_3$	65,79	8,07	5,90
IIb	65,5	169—170 (1)	1,0373	1,5196	67,31	8,48	5,84	$C_{14}H_{21}NO_3$	66,90	8,42	5,57
IIc	75,5	174—175 (3)	1,0359	1,5075	68,10	8,61	5,97	$C_{15}H_{23}NO_3$	67,89	8,73	5,27
IId	88,0	165—166 (2)	1,0374	1,5038	68,08	9,04	5,64	$C_{15}H_{23}NO_3$	67,89	8,73	5,27
IIe	74,3	154—155 (1)	1,0277	1,5034	69,17	9,11	5,55	$C_{16}H_{25}NO_3$	68,78	9,01	5,01
IIf	77,8	164—165 (2)	1,0222	1,5062	69,11	9,56	5,53	$C_{16}H_{25}NO_3$	68,78	9,01	5,01
IIg	65,8	159—160 (1)	1,0707	1,5138	66,90	8,10	6,05	$C_{14}H_{21}NO_3$	66,90	8,42	5,57
IIh	60,0	166—167 (1)	1,0470	1,5108	68,34	8,98	5,57	$C_{15}H_{23}NO_3$	67,89	8,72	5,27
IIi	56,7	174—175 (1)	1,0102	1,5181	69,80	8,20	5,43	$C_{16}H_{25}NO_3$	68,78	9,01	5,01
IIj	58,6	161—162 (2)	1,0265	1,5038	68,78	8,72	4,90	$C_{16}H_{25}NO_3$	68,78	9,01	5,01

TABLE 2. N-(Phenethyl)dihydrouracils (IIIa-j)

Compound	Yield, %	mp, °C	R_f	Found, %			Empirical formula	Calculated, %		
				C	H	N		C	H	N
IIIa	57,0	124—125	0,50	63,08	6,15	11,62	$C_{13}H_{16}N_2O_3$	62,88	6,49	11,28
IIIb	52,5	133—134	0,54	64,52	6,82	10,54	$C_{14}H_{18}N_2O_3$	64,10	6,91	10,45
IIIc	44,3	127—128	0,50	64,84	7,10	10,26	$C_{15}H_{20}N_2O_3$	65,19	7,29	10,13
IIId	45,3	137—138	0,59	64,87	7,46	10,36	$C_{15}H_{20}N_2O_3$	66,19	7,63	10,13
IIIe	58,6	117—118	0,65	66,53	8,01	10,08	$C_{16}H_{22}N_2O_3$	66,18	7,63	9,64
IIIf	61,1	115—116	0,67	66,56	8,02	10,03	$C_{16}H_{22}N_2O_3$	66,18	7,63	9,64
IIIg	42,0	121—122	0,54	64,01	6,80	10,96	$C_{14}H_{18}N_2O_3$	64,10	6,91	10,45
IIIh	45,1	115—116	0,59	65,50	7,82	10,11	$C_{15}H_{20}N_2O_3$	65,19	7,29	10,13
IIIi	34,5	116—117	0,57	65,78	7,80	10,03	$C_{16}H_{22}N_2O_3$	66,18	7,63	9,64
IIIj	64,1	105—106	0,61	65,79	7,88	10,07	$C_{16}H_{22}N_2O_3$	66,18	7,63	9,64

was carried out on Silufol UV-254 plates in the system ether—ethanol (1:0.06) for the dihydrouracils, and in ether for the dihydrothiouracils. The spots were visualized with an Ultrachemoscope UI-1.

Methyl β-(p-Alkoxyphenethylamino)- and β-(p-alkoxy-m-methylphenethylamino)propionates (IIa-j). A mixture of 0.05 mole of the p-alkoxyphenethylamine or p-alkoxy-m-methylphenethylamine, 4.6 g (0.05 mole) of methyl acrylate, and 30 ml of absolute methanol was kept at room temperature for 25-30 h. The methanol was distilled off, and the residue distilled in vacuo (Table 1).

N'-(Phenethyl)-5,6-dihydrouracils (IIIa-j). A mixture of 0.02 mole of the methyl ester (IIa-j), 6 g (0.1 mole) of urea, and 8 ml of glacial acetic acid was boiled for 3 h, then 5 ml of concentrated sulfuric acid was added slowly, followed by boiling for a further 2 h. The mixture was diluted with water (1:5), and the solid which separated on standing was filtered off, washed with water, and recrystallized from ethanol (Table 2).

N'-(Phenethyl)-5,6-dihydrothiouracils (IIIk-t). A mixture of 0.02 mole of the methyl ester (IIa-j), 3.8 g (0.05 mole) of ammonium thiocyanate, and 8 ml of glacial acetic acid was heated for 5 h at 100-105°C. Workup was as in the preceding example (Table 3).

EXPERIMENTAL BIOLOGY

The antitumor and antistaphylococcal activity of the test compounds was examined in white mongrel mice and rats of both sexes, weighing 18-20 and 90-110 g, respectively. In all, 860 mice and 350 rats were used.

The antitumor properties of (IIIa-t) were studied by standard methods [6]. Toxicities were determined by a single intraperitoneal administration to mice. The compounds were administered to the animals as suspensions in a 0.5% solution of carboxymethylcellulose. For each compound, the absolute lethal (LD_{100}) and maximum tolerated doses were determined. The LD_{100} values for the dihydrouracils were 500-1500 mg/kg, and for the dihydrothiouracils, 2500-3750 mg/kg. Introduction of a methyl group into the benzene ring had no marked effect on toxicity. The antitumor activities of the compounds were studied in doses of 1/15 and 1/20 of the LD_{100} in rats and mice with transplanted tumors (sarcomas 45 and 180, Walker's carcinosarcoma, and Ehrlich's ascitic carcinoma). The compounds were administered only daily for 8 days to rats, and for 6 days to mice. It was found that (IIIk-p) display moderate antitumor activity against sarcomas 45 and 180, and Walker's carcinosarcoma (Table 4).

TABLE 3. N-(Phenethyl)dihydrothiouracils (IIIk-t)

Compound	Yield, %	mp, °C	R_f	Found, %		Molecular formula	Calculated, %	
				N	S		N	S
IIIk	43,1	164—165	0,53	10,55	11,72	$C_{13}H_{16}N_2O_2S$	10,59	12,12
IIIl	24,8	161—162	0,60	10,13	11,39	$C_{14}H_{18}N_2O_2S$	10,06	11,51
IIIm	25,4	155—156	0,65	9,45	11,35	$C_{15}H_{20}N_2O_2S$	9,58	10,96
IIIn	21,3	171—172	0,68	9,95	10,68	$C_{15}H_{20}N_2O_2S$	9,58	10,96
IIIo	28,6	155—156	0,62	9,40	10,57	$C_{16}H_{22}N_2O_2S$	9,14	10,46
IIIp	22,0	181—182	0,66	9,44	10,16	$C_{16}H_{22}N_2O_2S$	9,14	10,46
IIIq	30,0	178—179	0,56	10,13	11,52	$C_{14}H_{18}N_2O_2S$	10,06	11,51
IIIr	29,5	155—156	0,60	9,29	10,49	$C_{15}H_{20}N_2O_2S$	9,58	10,96
IIIs	20,3	164—165	0,54	9,53	10,08	$C_{16}H_{22}N_2O_2S$	9,14	10,46
IIIt	25,0	173—174	0,66	8,83	10,58	$C_{16}H_{22}N_2O_2S$	9,14	10,46

TABLE 4. Antitumor Activity of Dihydrothiouracils (IIIk-p)

Compound	Dose, mg/kg	Inhibition of tumor growth, %*			
		rats		dose, mg/kg	Mice (sarcoma 180)
		sarcoma 45	Walker's carcinosarcoma		
IIIk	150	55	33	250	34
IIIl	150	53	35	250	0
IIIm	200	36	34	300	35
IIIn	150	0	31	250	0
IIIo	200	38	39	300	51
IIIp	200	36	34	300	45

*The results were statistically significant (P = 0.95).

Of the remaining compounds only (IIIc, d, h, i, s) displayed similar antiblastic activity, suppressing the growth of the tumors by 30-50%. None of the test compounds were active against Ehrlich's ascites carcinoma. It is noteworthy that in all the groups, activity was shown by compounds containing the propoxyphenethyl group.

In a model of generalized staphylococcal infection in white mice, induced by intraperitoneal infection [7], the chemotherapeutic effects of (IIIg-l, q-s) were examined. The compounds were administered in a single internal dose at the time of infection. Norsulfazole (serial No. 134,079) was used as the control drug. Following a single injection in a dose of 2500-3000 mg/kg, none of these compounds caused visible toxic effects in white mice. The compounds were not tested in higher doses. Following infection with strain 4-O, in doses of 1000 and 1500 mg/kg compounds (IIIi, l, q, r) had no therapeutic effects, (IIIg, h) in a dose of 1500 mg/kg increased the number of days of survival of the animals slightly in comparison with the untreated animals, and (IIIs) increased survival, increasing the number of days of survival of the treated animals by a significant 60%. In a dose of 1500 mg/kg, norsulfazol increased survival by 40%. Compound (IIIs) also slightly increased the survival of animals infected with other strains of Staphylococcus (91, Smith, 35), but to a smaller extent than norsulfazol. As with antitumor activity, here also the compound containing the propoxy group was outstanding (IIIs).

Also investigated were the antimutagenic effects of (IIIg-j, q-t) on biochemical strains of E. coli P-678 and Actinomyces rimosus 222, auxotrophic to threonine and lysine, respectively. The activity of the compounds was assessed from the frequency of occurrence of revertants from the auxotrophic to the prototrophic state at the loci responsible for the synthesis of threonine and lysine. The compounds were tested in doses of 100 mmole, the cultures being treated for 20 min. The controls were mutations which appeared spontaneously [8]. The effects of the same compounds on UV-induced mutations were examined in the same subjects. UV irradiation was effected with a BUF-30 bactericidal lamp at a distance of 60 cm from the irradiation source, at room temperature and with constant stirring for 90 sec. The experiments were carried out in a darkened box in red light [9]. In the combined treatment of the test subjects, one type of treatment was followed immediately by the other (UV irradiation + protector). The irradiated microorganisms were treated with the compounds in a dose of 10 mmole for 10 min. The number of mutations arising following treatment of the test subjects with UV served as the control.

The results for the protective effects of the dihydrouracils are shown in Table 5. Compounds (IIIg, h, j, s), while permitting a high survival rate, displayed antimutagenic effects, inducing E. coli revertants below the

TABLE 5. Antimutagenic Effects of Dihydrouracils and Thiouracils in E. coli and Actinomycetes

Compound	Effect on spontaneous mutations						Effect of UV-induced mutations		
	E. coli P=678 thr+			Act. rimosus 222 lys−					
	survival rate, %	revertants per 10 surviving cells		survival rate, %	revertants per 10^5 surviving spores		survival rate, %	revertants per 10^5 surviving spores	
		abs.	% of control		abs.	% of control		abs.	% of control
IIIg	88	5,7±0.4	71	173	1,2±0,1	60	112,5	1,3±0,25	44
IIIh	123	4,8±0.55	60	120	2,5±0,25	125	112,5	3,5±0,4	117
IIIi	40,5	9,8±1.2	120	123	1.65±0.2	81*	87,5	3,4±0,25	113
IIIj	119	5,8±0.65	73	156	2,5±0.35	125	119	2,5±0,2	83*
IIIq	128	8,5±0.9	106	113	3,5±0,4	176	106	2,8±0.3	93*
IIIr	138	7,2±0.6	90*	153	2,3±0,25	114	96	3,2±0,5	107
IIIs	130	6,0±0,7	76	110	2,3±0,3	114	87,5	3,4±0,4	113
IIIt	52	12,5±1.5	156	106	2,8±0,2	140	87,5	2,7±0,2	90*
Control	100	8,0±0.65	100	100	2,0±0,3	100	100	3,0±0,4	100

*Result statistically nonsignificant.

level of those occurring spontaneously by 24-40%. In the case of Actinomycetes, a statistically significant reduction in revertants was obtained with (IIIg) (40%). This compound also had a protectant effect in a study of UV-induced mutations in Actinomycetes, reducing the frequency of occurrence of mutations by 56%. In E. coli, the compounds showed no radioprotectant activity, and these results are not shown in Table 5.

These varying antimutagenic effects therefore encourage further investigations of N-substituted dihydrouracils and thiouracils with the object of discovering highly active protectants.

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