SEARCH FOR NEW DRUGS

SYNTHESIS AND ANTITUMOR ACTIVITY OF THE 6-*p*-ALKOXYPHENYL DERIVATIVES OF HEXAHYDROPYRIMIDINE-2,4-DIONES

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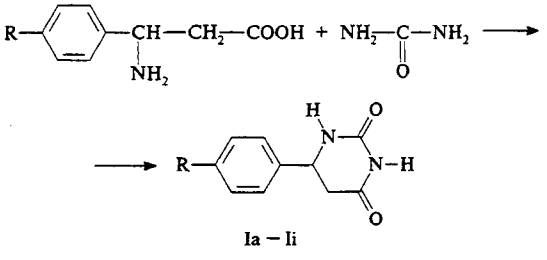
In continuation of our previous investigation into the relationship between the structure of the cyclic compounds containing –CO–NH–CO–NH-groups and their biological activity [1], we have synthesized new 6-*p*-alkoxyphenyl derivatives of hexahydropyrimidine-2,4-diones (Ia – Ii, IIc, IIe, IIf). Our interest in these compounds as inspired by the data indicating that hexahydropyrimidine-2,4-diones can be potential antitumor preparations [2 – 4]. An unsubstituted 6-phenylhexahydropyrimidine-2,4-dione (Ia) [5] and a series of its alkoxy derivatives (Ib – Ii) were synthesized in order to establish the relationship between their antitumor activity and the nature of substituents, while compounds IIc, IIe, and IIf were obtained in order to elucidate the role of the second substituent in position 6 of the pyrimidine ring.

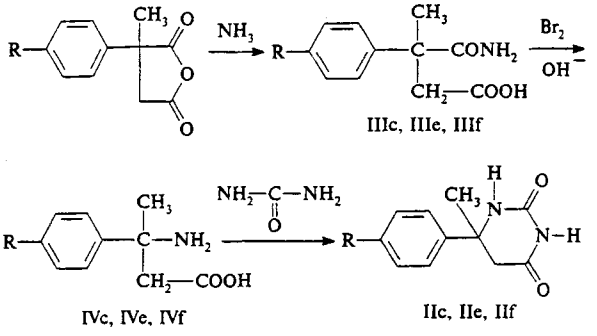
Compounds Ib – Ii were synthesized using a simplified variant of the conventional method [6] based on a single-stage condensation of substituted β-phenyl-β-alanines with urea:



Ia – Ii

R = H (a), CH_3O (b), C_2H_5O (c), *n*-C_3H_7O (d), *iso*-C_3H_7O (e), n-C_4H_9O (f), *iso*-C_4H_4O (g),*n*-$C_5H_{11}O$ (h), *iso*-$C_5H_{11}O$ (i).

Compounds IIc, IIe, and IIf were obtained according to the following scheme:



IIIc, IIIe, IIIf

IVc, IVe, IVf IIc, IIe, IIf

The initial anhydrides and amides of substituted succinic acids were synthesized as described previously [7]. The succinic acid amides (IIIc, IIIe, IIIf) were converted by the Hofmann rearrangement into 3-*p*-alkoxyphenyl-3-aminobutanoic acids (IVc, IVe, IVf). The latter compounds were identified by their mass spectra containing ion peaks with m/z = 164, 178, and 192 amu, respectively, suggesting the presence of $[M–CH_2COOH]^+$ fragments. Assignment of the mass peaks to these fragments was confirmed by analysis of the specially synthesized methyl ester of amino acid IVe, the mass spectrum of which contained a peak with m/z = 164 corresponding to the $[M–CH_2COOCH_3]^+$ fragment. Cyclization of compounds IVc, IVe, and IVf with urea led to the corresponding derivatives IIc, IIe, and IIf.

EXPERIMENTAL CHEMICAL PART

TLC was performed on a supported silica gel (KSK grade gypsum) eluted with a phenol – *p*-xylene – formic acid (3 : 1 : 2) mixture and developed by Bromophenol Blue. The IR absorption spectra were measured on an UR-20 spectrophotometer (Germany) using samples prepared as nujol mulls. The mass spectra were obtained on an MX-1320 spectrometer. The positions of molecular peaks agree with the

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empirical formulas proposed. The 1H NMR spectra were obtained on a Varian T-60 spectrometer (USA) using TMS as the internal standard. The data of elemental analyses agree with the results of analytical calculations.

Physicochemical characteristics and yields of the synthesized compounds are listed in Table 1.

6-*p*-Ethoxyphenylhexahydropyrimidine-2,4-dione (Ic). A mixture of 2 g (10 mmole) of β-*p*-ethoxyphenyl-β-alanine [5] and 1.8 g (30 mmole) of urea was heated to 145–150°C for 30 min. Upon cooling, the solidified reaction mass was dissolved in a 10% aqueous NaOH. The solution was decolorized by activated charcoal and filtered. The filtrate was acidified by adding hydrochloric acid to pH 3. The precipitated crystals were filtered, washed with cold water, and recrystallized from ethanol. IR spectrum of compound Ic (ν_{max}, cm^{-1}): 3460, 3320 (NH), 1720, 1700, 1760 (C=O); 1H NMR spectrum in DMSO-d_6 (δ, ppm): 1.30 (t, 3H, CH_3), 2.63 (d, 2H, CH_2), 3.95 (q, 2H, CH_2O), 4.93 (q, 1H, CH), 6.83–7.26 (m, 4H, C_6H_4), 7.90 (s, 1H, 3-NH), 10.2 (s, 1H, 1-NH).

Similar procedures were used to obtain compounds Ib–If, IIc, IIe, and IIf.

3-Amino-3-*p*-ethoxyphenylbutanoic acid (IVc). To 25 g (100 mmole) of compound IIIc was added a freshly prepared solution of potassium hypobromite (120 mmole of bromine and 600 mmole of potassium hydroxide in 200 ml of water), and the mixture was stirred at 0°C until complete dissolution of the residue. Then the solution was heated to 60°C for 1 h, cooled to room temperature, clarified by activated charcoal, and neutralized by adding hydrochloric acid pH 6.5–7.0. The precipitated crystals were filtered, washed with water, dissolved in 50 ml of 10% aqueous HCl, and alkalized by gradually adding solid potassium hydroxide. The precipitate formed at pH 6.5 was removed by filtration. The filtrate was continuously alkalized to pH 7.0 to obtain the precipitate of compound IVc. Compounds IVe and IVf were obtained similarly.

TABLE 1. Physicochemical Characteristics of the Synthesized Compounds

Compound	Yield, %	M.p., °C	R_f	Empirical formula
Ib	85.2	216–217	0.68	$C_{11}H_{12}N_2O_2$
Ic	86	222–223	0.68	$C_{12}H_{14}N_2O_3$
Id	82	203–204	0.67	$C_{13}H_{16}N_2O_3$
Ie	87	150–151	0.68	$C_{13}H_{16}N_2O_3$
If	73.5	199–200	0.68	$C_{14}H_{18}N_2O_3$
Ig	68.9	170–172	0.68	$C_{14}H_{18}N_2O_3$
Ih	79.2	260–261	0.68	$C_{15}H_{20}N_2O_3$
Ii	73.6	175–176	0.68	$C_{15}H_{20}N_2O_3$
IIc	44	228–229	0.54	$C_{13}H_{16}N_2O_3$
IIe	70	216	0.50	$C_{14}H_{18}N_2O_3$
IIf	75	162–163	0.80	$C_{15}H_{20}N_2O_3$
IVc	53	253–254	0.34	$C_{12}H_{17}NO_3$
IVe	72	156–158	0.61	$C_{13}H_{19}NO_3$
IVf	64	272	0.47	$C_{14}H_{21}NO_3$

EXPERIMENTAL BIOLOGICAL PART

The acute toxicity of the synthesized compounds upon a single intraperitoneal injection was studied by the conventional method on white mice weighing 17–20 g [8]. The compounds were prepared *ex tempore* as suspensions in a

TABLE 2. Acute Toxicity and Antitumor Activity of 6-Phenylhexahydropyrimidine-2,4-dione and Its Derivatives

Compound	R	LD_{100}, mg/kg	MPD, mg/kg	Dose, mg/kg	Sarcoma 45		Walker carcinosarcoma		Schvetz leukemia		Dose, mg/kg	Sarcoma 180		Ehrlich carcinoma, survival lifetime gain, %
					T, %	K_g, %	T, %	K_g, %	T, %	K_g, %		T, %	K_g, %	
Ia	H	90	40	5	Stimulation						10	0	„	
Ib	CH_3O	3000	2250	150	0**		15*	+4.2	0		300	52	+1.6„	
Ic	C_2H_5O	1500	1200	75	58	−2.5	28.5	+10.9	45.6	−5.2	150	50	18	32
Id	*n*-C_3H_7O	1500	1000	70	0		0		0		150	0		0
Ie	*iso*-C_3H_7O	400	275	20	0		0		0		40	25	+6.5	0
If	*n*-C_4H_9O	1500	1250	70	0		0		0		150	0		0
Ig	*iso*-C_4H_9O	700	500	40	0		0		0		60	Stimulation		0
Ih	*n*-$C_5H_{11}O$	2500	2000	125	0		0		0		250	0		0
Ii	*iso*-$C_5H_{11}O$	1500	1000	70	0		0		0		150	0		0
IIc	C_2H_5O	4500	–	220	64	−4.5	0		0		450	47	+5.0	0
IIe	*iso*-C_3H_7O	4000	–	200	70	−3.0	0		0		400	41	+0.9	0
IIf	*n*-C_4H_9O	2500	–	125	66	−4.5	0		0		250	46	−2.4	0
5-Fluoro-uracil		200	75	10	32	−7.3	30	−5.6	49	−6.8	25	46	−11.0	39

* $\alpha \leq 0.95$;
** No activity.

0.5% carboxymethyl cellulose solution. During the test, the animals were kept on a mixed diet under usual laboratory conditions.

The antitumor activity was studied on rats with sarcoma 45, Walker carcinosarcoma, or Schvetz leukemia, and on mice with sarcoma 180 or the Ehrlich ascite carcinoma. The animals were treated with test preparations injected intraperitoneally at a dose of 1 / 20 LD_{100} for rats, and 1 / 10 LD_{100} for mice. The therapeutic effect of each compound was evaluated by the degree of tumor growth inhibition (T, %); for the Ehrlich ascite carcinoma, the effect was evaluated by the percentage increase in the survival lifetime of the ascite mice against the control. The general toxicity was judged by the growth coefficient (K_g, %). The reference compound was 5-fluorouracil.

The experimental data were statistically processed using the Student – Fisher method; the results were considered reliable for $\alpha \geq 0.95$.

The results of our experiments with the series of synthesized 6-*p*-alkoxyphenylhexahydropyrimidine-2,4-dione derivatives showed that unsubstituted 6-phenylhexahydropyrimidine-2,4-dione (Ia) is a toxic compound (MPD = 40 mg / kg) possessing no significant antitumor properties (Table 2). The first derivative in the homologous series studied, 6-*p*-methoxyphenylhexahydropyrimidine-2,4-dione (Ib) has a low toxicity and efficiently inhibits only the growth of sarcoma 180 (T = 52%), showing no general toxic effect upon the organism of test mice (K_g = 1.6%). Compound Ic with an ethoxyphenyl radical exhibits antitumor activity with respect to both solid tumors and the Ehrlich ascite carcinoma. This low-toxicity compound (MPD = 1200 mg / kg) inhibits the growth of sarcoma 45 (T = 58%), moderately suppresses the Schvetz leukemia (T = 45%) and sarcoma 180 (T = 50%), and shows no effect with respect to the Walker carcinosarcoma. Test mice with the Ehrlich ascite carcinoma, treated with compound Tc, exhibited a reliable increase in the survival lifetime (32%).

The hexahydropyrimidine-2,4-dione derivatives with propoxy (Id), isopropoxy (Ie), butoxy (If), isobutoxy (Ig), amyloxy (Ih), and isoamyloxy (Ii) radicals in the benzene ring showed no significant antitumor efficiency. Therefore, introduction of a bulky alkyl radical leads to the loss of the antitumor activity. It should be also noted that compounds with iso-alkoxy radicals (Ie, Ig, Ii) exhibit a higher toxicity compared to that of the derivatives with *n*-alkyl radicals: the former are characterized by MPD = 1000 – 275 mg / kg, while the latter have MPD = 2000 – 1000 mg / kg.

The antitumor effect of compounds Ib – Ii depends on the character of the alkoxy radical in the benzene ring. Compound Ic is superior to 5-fluorouracil with respect to the therapeutic effect upon sarcoma 45, and has comparable activity with respect to the Schvetz leukemia, Walker carcinosarcoma, sarcoma 180, and Ehrlich ascite carcinoma. Unlike 5-fluorouracil, compound Ic produces no general toxic effect on the organism of mice with sarcoma 180. Compound Ib and 5-fluorouracil also showed equal activities with respect to sarcoma 180.

In order to elucidate the influence of the second substituent in the molecule of pyrimidinedione on its antitumor activity. we have studied 6-methyl-6-*p*-methoxyphenylhexahydropyrimidine-2,4-diones IIc, IIe, and IIf. Therapeutic doses of these derivatives produced reliable inhibition of the growth of sarcoma 45 (T = 64 – 70%) and sarcoma 180 (T = 41 – 46%), but produced no significant suppressive effect with respect to the Walker carcinosarcoma, Schvetz leukemia, and Ehrlich ascite carcinoma.

These results indicate that compounds IIc, IIe, and IIf are somewhat more active than compounds Ib – Ii with respect to sarcoma 45. This is probably related to the presence of a CH_3 group as the second substituent in position 6 of the pyrimidine ring. As compared to 5-fluorouracil, compounds IIc, IIe, and IIf exhibit lower toxicity, are superior with respect to sarcoma 45, and show comparable action upon sarcoma 180.

Thus, the results of our investigation suggest that the search for new antitumor drugs among the alkoxyphenyl derivatives of hexahydropyrimidine-2,4-diones may have good prospects.

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