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SYNTHESIS AND ANTITUMOR PROPERTIES OF 1,3-DIAZA-2-PHOSPHAADAMANTANE DERIVATIVES, PHOSPHORYL-CONTAINING 3,7-DIAZABICYCLO[3.3.1]NONANE, AND 1,3-DIAZAADAMANTANE[1]

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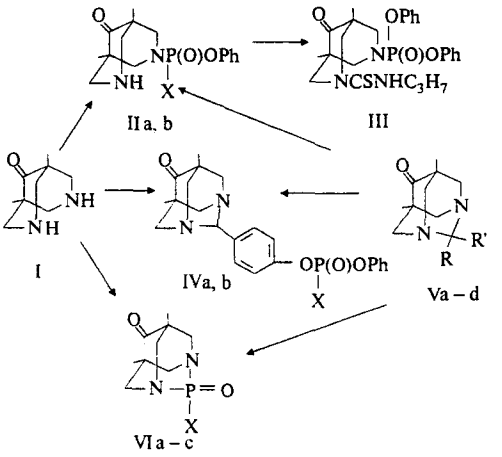
In the search for new antitumor compounds, we synthesized a number of 3,7-diazabicyclo[3.3.1]nonanes and 1,3-diazaadamantanes containing a phosphoryl group, and compounds derived from the previously unknown 1,3-diaza-2-phosphaadamantane. We used phenoxy- and *bis*(2-chloroethyl)amino groups as substituents in the phosphoryl group. 1,5-Dimethyl-9-oxo-3,7-diazabicyclo[3.3.1]nonane (I) [1] and 5,7-dimethyl-6-oxo-1,3-diazaadamantanes (Va – d) [2] were used as starting compounds; some of their derivatives exhibited antitumor activity in the experiments [3, 4].

3-Phosphoryl-substituted 3,7-diazabicyclo[3.3.1]nonanes (IIa, b) were synthesized in the reaction of diazabicyclononane I with diphenyl phosphorochloridate and phenyl-*bis*(2-chloroethyl)-phosphoramidochloridate [5] (in the ratio 1 : 1). The excess of diphenoxyphosphoryl chloride (1 : 2) did not lead to the formation of the corresponding 3,7-disubstituted derivative of 3,7-diazabicyclononane, although we showed that diazabicyclononane IIa readily reacted with propyl isothiocyanate to form the mixed 3,7-disubstituted derivative of 3,7-diazabicyclononane (III). The interaction of 1,3-diazaadamantanes containing unsubstituted (Va), C-mono- (Vc), and the C,C-disubstituted (Vb) methylenediamine group with diphenoxyphosphoryl chloride also leads to diazabicyclononane IIa.

2-Phenyl-1,3-diazaadamantane derivatives with a substituted phosphoryl group on the benzene ring (IVa, b) were synthesized by condensation of diazabicyclononane I with 4-formylphenyl diphenyl phosphate or phenyl-*bis*(2-chloroethyl)-phosphoramidate [the last-mentioned compounds were obtained from sodium 4-formylphenolate and diphenoxyphosphoryl chloride or phenyl-*bis*(2-chloroethyl)-phosphoramidochloridate and introduced into condensation without preliminary isolation]. Diazaadamantane IVa was obtained by an alternate synthesis for identification from 2-(4-hydroxyphenyl)-5,7-dimethyl-6-oxo-1,3-diazaadamantane (Vd) and the diphenyl ester of phosphorochloridic acid.



II, IV, VI: X = OPh (a), $N(CH_2CH_2Cl)_2$ (b), Cl (c).

V: R = R′ = H (a), R = R′ = Me (b), R = H, R′ = Et (c), R = H, R′ = C_6H_4OH-*p* (d).

1,3-Diaza-2-phosphaadamantanes (VIa – c) were synthesized in the reaction of diazabicyclononane I with the phenyl ester, N,N-*bis*(2-chloroethyl)amide, and chloride of phosphorodichloridic acid. In addition, we found that the interaction of diazaadamantane Va with phenyl phosphorodichloridate and $POCl_3$ also results in formation of diazaphosphaadamantane VIa and VIc, respectively (reaction of diazaphosphaadamantane VIc with sodium phenolate gives diazaphosphaadamantane VIa). Repeated experiments

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showed that the best yield of diazaphosphaadamantane VIa is observed when the reaction is carried out in an organic solvent at room temperature for 4 h with 20% excess of phenyl phosphorodichloridate with respect to diazaadamantane in the presence of triethylamine or pyridine (3-fold amount with respect to the dichloride). 1,3-Diazaadamantanes containing a C-mono-(Vc) or C,C-disubstituted methylenediamine group (Vb) react with phenyl phosphorodichloridate in a similar manner to form diazaphosphaadamantane VIa. Therefore, in the above-mentioned cases the breaking of N–C bonds in the methylenediamine or in the C-substituted methylenediamine group of the diazaadamantane ring results in formation of a diazabicyclononane derivative (in the case of diphenoxyphosphoryl chloride) and a diazaphosphaadamantane derivative (as a result of the following closure of the diazabicyclononane ring) in the cases of phenyl phosphorodichloridate and $POCl_3$.

The structure of the compounds synthesized was established by elemental analysis and IR, PMR, and mass-spectra. The purity was determined by TLC.

We have studied the antitumor activity of both synthesized (II – IV, and VI) and starting compounds (I and V).

According to the data presented in Table 1, compound I · HCl without substituents at the nitrogen atoms is toxic and exhibits a weak activity only against sarcoma 45. Diazabicyclononane derivatives IIa and IIb are less toxic; compound IIa exhibits weak antitumor activity with respect to sarcomata 45 and 180, and stimulates the growth of Shvets leukemia; and compound IIb, which contains a *bis*(2-chloroethyl)amine group instead of one of the phenoxy groups, is not active. Introduction of a propylthiocarbamoyl group at the second nitrogen atom of compound IIa lowered the toxicity of the obtained compound III for the same antitumor activity.

The study of 1,3-diazaadamantanes showed that compounds Va · HCl, Vb, and Vc were slightly toxic, and compound Vd was not toxic. Compounds IVa and IVb, obtained by the introduction of a substituted phosphoryl group into compound Vd, are also nontoxic. Of the 1,3-diazaadamantane derivatives, only 1,3-diazaadamantane Va · HCl, containing an unsubstituted group, exhibited a slight antitumor effect against sarcomata 45 and 180.

The 1,3-diaza-2-phosphaadamantanes VIa and VIb are slightly toxic substances. Compound VIa with a phenoxy group exhibited comparatively higher antitumor activity than did compound VIb (with a *bis*(2-chloroethyl)amine group) with respect to the tumor strains. Thus, compound VIa showed 52% reliable inhibition of the growth of sarcoma 45 and did not exert a toxic effect on the tested animals. Compound VIa was active against this strain even in oral administration. Under the same conditions, compound VIb inhibited the growth of sarcoma 45 by 35%. Compound VIa inhibited the growth of sarcoma 180 by 42% ($\alpha > 0.98$). The efficacy of VIb against this strain was 33.6% ($\alpha > 0.95$). Both compounds do not induce general toxic phenomena, namely a sharp decrease in the mass of tested mice.

All the compounds synthesized have no antitumor effect with respect to Shvets leukemia, Walker carcinosarcoma, and ascitic Ehrlich carcinoma.

Thus the derivatives of 1,3-diaza-2-phosphaadamantane are comparatively more active than the derivatives of 3,7-diazabicyclononane and 1,3-diazaadamantane.

TABLE 1. Toxicity and Antitumor Activity of Compounds I – VI

Compound	LD_{100}, mg/kg	MTD, mg/kg	Dose, mg/kg/day	Sarcoma 45 T, %	Sarcoma 45 K_g, %	Dose, mg/kg/day	Sarcoma 180 T, %	Sarcoma 180 K_g, %
I · 2HCl	300	150	18	38.5	+14.0	30	Inactive	
IIa	550	200	25	34	−6.0	50	31	−2.1
IIb	500	150	25	Inactive		50	–	
III	5000	–	250	47	−9.0	500	26	−3.4
VIa	4000	3000	150	30.5	−9.9	300	–	
IVb	5000	–	250	Inactive		500	–	
Va · HCl	1100	800	50	52	−5.0	100	33	+2.6
Vb	1100	900	50	Inactive		100	–	
Vc	1250	1000	60	–"–		125	–	
Vd	5000	–		250	–"–		500	–
VIa	2500	2000	120	52.5	+6.1	250	42	−1.7
			120*	33.3	+6.0			
			240*	48	−9.1			
VIa	1000	850	50	35	−5.7	100	33.6	+9.0

* Oral administration.

CHEMICAL EXPERIMENTAL PART

IR spectra were recorded on a Specord UR-20 (DDR) spectrometer using Vaseline. PMR spectra were obtained on a Varian T-60 (USA) spectrometer with TMS used as the internal standard. Mass-spectrometric determination of molecular masses was carried out on a MKh-1320 spectrometer (USSR) which provided for direct injection of the sample into the ion source. The energy of the ionizing electrons was 60 eV. The course of the reaction and the purity of the compounds were monitored by TLC on Silufol UV-254 plates in water – propanol systems, 7 : 3 (*A*) and 1 : 4 (*B*). Ninhydrin was used as the visualizing agent.

The elemental analysis data for compounds II – IV, and VI for C, H, N, P, and Cl match the calculated values.

1,5-Dimethyl-3-(diphenoxyphosphoryl)-9-oxo-3,7-diazabicyclo[3.3.1]nonane (IIa). *A*. To a solution of I (1.68 g, 10 mmole) in 100 ml of benzene diphenoxyphosphoryl chloride (2.7 g, 10 mmole) and Et_3N (1.0 g, 10 mmole) were added; the mixture was stirred for 2 h and filtered. The filtrate was washed with water, concentrated under vacuum, and the residue was recrystallized from heptane. Product IIa (2.6 g, 64.9%) was obtained, m. p. 123 – 125 °C (from heptane), R_f = 0.6 (*A*). PMR ($CDCl_3$) (δ, ppm): 0.9 (6H, s, $2CH_3$); 2.75 (2H, d, 2,4-CH, J = 13 Hz); 2.9 (1H, s, NH); 3.15 (2H, d, 6,8-CH_a), J = 13 Hz); 3.3 (2H, d, 6,8-CH_e, J = 13 Hz); 4.0 (2H, d, 2,4-CH, J = 13 Hz, J = 6 Hz); 7.15 – 7.4 (10H, m, arom. protons). M^+ 400.

B. A mixture of 1 mmole of diazaadamantanes Va – c, 0.3 g (1 mmole) of diphenoxyphosphoryl chloride, and 0.2 g (2 mmole) Et_3N in 30 ml of acetonitrile was refluxed for 4 h and filtered; the filtrate was distilled under vacuum, the residue was dissolved in 15 ml of ethyl acetate; the solution prepared was washed with water, dried over $CaCl_2$, and distilled. The yields of compound IIa were 45, 45, and 42%, respectively, m. p. 123 – 125 °C, $R_f = 0.6$ (*A*).

1,5-Dimethyl-3-[N,N-*bis*(2-chloroethyl)aminophenoxy phosphoryl]-9-oxo-3,7-diazabicyclo[3.3.1]no- nane (IIb). A mixture of I (1.7 g, 10 mmole), phenyl N,N-*bis*(2-chloroethyl)phosphoramidochloridate (3.16 g, 10 mmole), and Et_3N (1.0 g, 10 mmole) in 100 ml of benzene was refluxed for 4 h and filtered. The filtrate was washed with water (2 × 50 ml), dried over Na_2SO_4, evaporated under vacuum, the residual oil was crystallized by petroleum ether. Compound IIb (3.1 g, 68%) was obtained, m. p. 119 – 212 °C, $R_f = 0.62$ (*A*). M^+ 451.

1,5-Dimethyl-3-(diphenoxyphosphoryl)-7-(propylthio carbamoyl)-9-oxo-3,7-diazabicyclo[3.3.1]no- nane (III). *A.* A mixture of IIa (2 g, 5 mmole) and propyl isothiocyanate (0.5 g, 5 mmole) in 50 ml of benzene was refluxed for 2 h and cooled. The crystals precipitated were filtered off and crystallized from ethanol. Product III (2.2 g, 87.6%) was obtained, m. p. 218 – 220°C (from ethanol), $R_f = 0.88$ (*A*). PMR (DMSO-d_6) (δ, ppm): 0.7 (3H, distorted t, CH_3); 0.9 (6H, s, $2CH_3$); 1.35 (2H, m, CH_2); 2.85 – 3.4 (8H, m, $4CH_2$N-ring); 3.8 (2H, m, NCH_2); 4.8 (1H, br. s, NH); 7.0 – 7.5 (10H, m, arom. protons). M^+ 501.

5,7-Dimethyl-6-oxo-2-[4-(diphenoxyphosphoryloxy) phenyl]-1,3-diazaadamantane (IVa). *A.* Diphenoxyphosphoryl chloride (5.4 g, 20 mmole) was added to a suspension of sodium 4-formylphenolate (2.88 g, 20 mmole) in 100 ml of acetonitrile for 4 h with stirring, and the resulting mixture was filtered. The filtrate was evaporated under vacuum; to the residue dissolved in 100 ml of ethanol, 3.34 g (20 mmole) of I was added and the mixture was refluxed for 2 h and cooled. The precipitate was filtered off and crystallized from ethanol to yield IVa (8 g, 79.3%), m. p. 145 – 147 °C (from ethanol), $R_f = 0.8$ (*A*). PMR ($CDCl_3$) (δ, ppm): 0.7 (3H, s, CH_3); 0.85 (3H, s, CH_3); 2.75 (2H, d, J = 13 Hz, $2CH_aN$); 3.2 – 3.6 (6H, m, $2CH_aN$, $4CH_eN$); 5.1 (1H, s, CH); 7.1 – 7.7 (14 H, m, arom. protons). M^+ 504.

B. A solution of sodium ethylate [prepared from 0.46 g (20 mmole) of sodium and 10 ml of ethanol] was added to a solution of Vd (5.44 g, 20 mmole) in 50 ml of absolute ethanol. The resulting mixture was allowed to stand for 3 h, the ethanol was distilled off, and 50 ml of acetonitrile was added; then a solution of 5.37 g (20 mmole) of diphenoxyphosphoryl chloride in 20 ml of acetonitrile was added dropwise with stirring. The resulting mixture was stirred for 3 h, refluxed for 30 min, and the solvent was distilled off under vacuum. Recrystallization of the residue from ethanol yields 9.1 g (90%) of IVa, m. p. 146 – 147 °C, $R_f = 0.8$ (*A*).

2-{4'-[N,N-*bis*(2-chloroethyl)aminophenoxyphospho- ryloxy]phenyl}-5,7-dimethyl-6-oxo-1,3-diaza- adamantane (IVb). Phenyl N,N-*bis*(2-chloroethyl)phosphoramidochloridate (6.32 g, 20 mmole) was added to a suspension of sodium 4-formylphenolate (2.88 g, 20 mmole) in 100 ml of acetonitrile for 4 h with stirring. The obtained mixture was filtered, the filtrate was concentrated under vacuum, the residue was dissolved in 100 ml ethanol, and compound I (3.34 g, 20 mmole) was added. The mixture was refluxed for 2 h and cooled. The precipitate formed was filtered off and recrystallized from ethanol. Product IVb (5 g, 90.5%) was obtained, m. p. 101 – 103 °C, $R_f = 0.7$ (*A*). IR spectrum (ν, cm^{-1}): 1280 (P–O–C), 1380 (P=O), 1580 (C–C arom.), 1700 (C = O). PMR ($CDCl_3$) (δ, ppm): 0.7 (3H, s, CH_3); 0.92 (3H, s, CH_3); 2.75 (2H, d, J = 13 Hz, $2CH_aN$); 3.1 – 3.7 (6H, m, $2CH_aN$, $4CH_eN$); 3.3 (4H, m, $2CH_2N$); 3.6 (4H, m, $2CH_2Cl$); 5.1 (1H, s, CH); 7.13 – 7.7 (9 H, m, arom. protons). M^+ 555.

5,7-Dimethyl-2,6-dioxo-2-phenoxy-1,3-diaza-2-phos phaadamantane (VIa). *A.* Triethylamine (1.0 g, 10 mmole) was added dropwise to a mixture of I (0.85 g, 5 mmole) and phenyl phosphorodichloridate (1.05 g, 5 mmole) in 20 ml of anhydrous benzene for 30 min, stirred for 2 h, and filtered. The filtrate was concentrated under vacuum; the residue was washed with water (10 ml), filtered off, and recrystallized from ethanol to yield VIa (0.8 g, 52.3%), m. p. 194 – 195 °C (from ethanol), $R_f = 0.6$ (*B*). IR spectrum (ν, cm^{-1}): 1280 (P–O–C), 1330 (P = O), 1600 (C–C arom.), 1710 (C = O). PMR ($CDCl_3$) (δ, ppm): 0.93 (6H, s, $2CH_3$); 2.97 (2H, d, J = 13 Hz, $2CH_aN$); 3.36 (2H, d, J = 13 Hz, $2CH_aN$); 3.94 (2H, d, $2CH_eN$); 4.22 (2H, d, J = 13 Hz, $2CH_eN$); 7.4 (5H, m, arom. protons). M^+ 306.

B. Triethylamine (0.94 g, 9 mmole) was added to a solution of Va (0.45 g, 2.5 mmole) in 50 ml of benzene, then phenyl phosphorodichloridate (0.63 g, 3 mmole) was added with stirring, and the resulting mixture was stirred for 4 h and filtered. The filtrate was washed with water, concentrated under vacuum, and the residue was crystallized from ethanol. Product VIa (0.64 g, 83.6%) was obtained, m. p. 194 – 195 °C (from ethanol), $R_f = 0.6$ (*B*).

C. Compound VIa was obtained by the same procedure from 0.21 g (1 mmole) of Vb or Vc, 0.21 g (1 mmole) of phenyl phosphorodichloridate, and 1 g (10 mmole) Et_3N. The yields of VIa were 0.18 g (60%) and 0.2 g (66.6%), respectively, m. p. 194 – 195 °C (from ethanol), $R_f = 0.6$ (*B*).

D. Freshly distilled $POCl_3$ (1.5 g, 10 mmole) was added to a solution of I (1.68 g, 10 mmole) or Va (1.8 g, 10 mmole) in 50 ml of dry benzene; then Et_3N(2.8 g, 20 mmole) was added with stirring, and the resulting mixture was stirred for 2 h and filtered. The filtrate was washed with water and concentrated under vacuum; the residue was washed with water and recrystallized from ethanol to yield VIc [1.4 g (56.5%) and 0.5 g (20.1%), respectively], m. p. 158 – 159 °C, $R_f = 0.7$ (*A*). A solution of VIc (2 g, 7.5 mmole) in 10 ml of acetonitrile was added to a solution of 1.16 g (10 mmole) of sodium phenolate in 100 ml of acetonitrile over the course of 30 min, then the

mixture was stirred for 4 h and filtered; the filtrate was concentrated under vacuum, the residue was washed with water and recrystallized from ethanol. Product VIa (2.3 g, 79.3%) was obtained, m. p. 194 – 195 °C (from ethanol), $R_f = 0.6$ (*B*).

2-*bis*(2-Chloroethyl)amino-5,7-dimethyl-2,6-dioxo-1, 3-diaza-2-phosphaadamantane (VIb). VIb was prepared by a procedure similar to that used for VIa (procedure *A*) from I (0.84 g, 5 mmole), *bis*(2-chloroethyl)amide of phosphorodichloridic acid (1.3 g, 5 mmole), and Et_3N (1 g, 10 mmole). The yield of VIb was 1.1 g (62.1%), m. p. 145 – 146 °C (from hexane), $R_f = 0.7$ (*A*). IR spectrum (ν, cm^{-1}): 1330 (P = O), 1700 (C = O). UV spectrum (96% ethanol), λ_{max} (ε), nm: 204 (1150), 238 (1240). M^+ 357.

BIOLOGICAL EXPERIMENTAL PART

The antitumor activity of the synthesized and starting compounds was studied by the procedure reported in [6] in rats with grafted sarcoma 45, Shvets leukemia, Walker carcinosarcoma, and in mice with grafted sarcoma 180, 37, and ascitic Ehrlich carcinoma. A therapeutic dose of every compound was determined by the acute toxicity (LD_{100} and MTD) of the compound in white mongrel mice weighing 17 – 21 g in intraperitoneal administration (1/20 and 1/10 LD_{100} for rats and mice, respectively). The substances were administered to animals intraperitoneally: IIa, Vb – d, VIa, b as a suspension in a 0.5% solution of carboxymethylcellulose; I, IIb, IIIa, b, and Va – in isotonic NaCl solution (dissolved previously in 0.1 ml of 96% ethanol). In the course of experiments the animals were kept under conventional laboratory conditions on a basic mixed diet. Active compounds were also tested for oral administration.

The therapeutic effect was estimated by the percent of inhibition of the tumor growth (*T*, %) or by an increase in the lifespan of tested mice with ascitic in comparison with the control. The toxic effect was characterized by the growth coefficient (K_g, %). The results of the experiments were treated statistically. The data are reliable with ($\alpha \geq 0.95$).

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