The IR spectra of the samples were recorded as mulls in vaseline oil with a UR-20 spectrometer.

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SYNTHESIS AND ANTIBLASTIC ACTIVITY OF SOME DERIVATIVES OF 1-PHENYL-3,3-DIMETHYLTRIAZENE

A. I. Kravchenko, V. A. Chernov,
V. G. Voronin, I. D. Petrova,
A. N. Leksin, N. F. Karaseva,
and V. P. Zelenova

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Literature data indicate that triazene-containing imidazole derivatives, for example 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxyamide (NSC 45 388, DTIC, or DIC) and 5-[3,3-bis(2-chloroethyl)-1-triazeno]imidazole-4-carboxamide (NSC 82 196 or BTIC) have found use in medical practice as antitumor drugs [1, 2]. There are also a number of communications about aromatic triazene derivatives which exert a cytostatic action [3-5]. Thereupon it is noted that the very active 1-(4-carbomethoxyphenyl)-3,3-dimethyltriazene is not inferior to DTIC in its antitumor effect. In distinction from heterocyclic triazenes, the synthesis of benzoic acid derivatives is far simpler, and the substituted triazenes obtained are more stable.

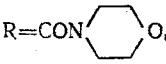
The method for preparing derivatives of 1-phenyl-3,3-dimethyltriazene is not complex, and reduces to diazotizing derivatives of p-aminobenzoic acid with sodium nitrite in the presence of a mineral acid, with subsequent reaction with appropriate amines upon addition of substances having a basic character, soda or sodium hydroxide, for example, at a temperature from −5 to 5°C [4-7]:

$$4{=}RC_6H_4NH_2 \rightarrow 4{=}RC_6H_4\overset{+}{N}{\equiv}N \rightarrow 4{=}RC_6H_4N{=}NN(CH_3)R',$$

S. Ordzhonikidze All-Union Scientific-Research Institute of Pharmaceutical Chemistry, Moscow. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 10, No. 11, pp. 72-75, November, 1976. Original article submitted May 24, 1976.

where

R=COOH, R′=CH_3 (I); R=CH_3O, R′=CH_3 (II);
R=C_2H_5O, R′=CH_3 (III); R=$COOCH_3$, R′=CH_3 (IV);
R=$COOC_2H_5$, R′=CH_3 (V): R=$COOCH(CH_3)_2$, R′=CH_3 (VI);
R=$COOCH_3$R′=H (VII); R=$CONH_2$, R′=CH_3 (VIII);
R=$CONHCH_3$, R′=CH_3 (IX); R=$CON(CH_3)_2$, R′=CH_3 (X);



For comparative study of antitumor activity, we synthesized compounds I-VIII, and also, additionally, derivatives of 1-(4-benzamido)-3,3-dimethyltriazene (VIII), since it is known that they give a definite cytostatic effect [5].

The 1-phenyl-3,3-dimethyltriazene derivatives prepared were studied for antiblastic activity and toxicity. Experiments were conducted on white mongrel mice (18-20 g in weight) and rats (100-120 g in weight) of both sexes, and on male mice of the DBA line (all told, in the experiments we used 1000 rats and about 1000 mice). The substances tested were introduced orally in vegetable oil and intraperitoneally in the form of a 10% alcoholic suspension. Antiblastic activity was studied in experiments on rats (Jensen sarcoma and sarcoma 45) and on mice (sarcoma 180 and L-1210 leukemia). Injections were started from the third to sixth day (solid tumors) or on the first day (L-1210 leukemia) after transplantation of the tumor and were continued daily for 4-7 days. The duration of observation was 12-15 days from the moment of tumor inoculation.

Effect on the growth of subcutaneous sarcomas was judged from the retardation index (I_m, in %), as defined by the formula

$$I_m = \frac{(W_C - W_O)100}{W_C},$$

where W_C and W_O are the mean weights of the tumor in control and test animals, respectively. An I_m with the sign (+) indicates retardation; one with the sign (—) indicates stimulation of the growth of the tumor. In the case where the difference between the mean weights of tumors in groups being compared was statistically insignificant, I_m was set equal to zero. The overall action of a substance on the organism of animals was evaluated from the state of the animals, the number of fallen individuals, and also from the growth index (K_g, in %), which reflects the weight change of the animals in the test group during the time of the experiment as compared with the control group. K_g was calculated by the formula

$$K_g = \frac{C_O \cdot A_C \cdot 100}{A_O \cdot C_C} - 100,$$

where A_O and A_C are the mean weights at the start of the experiment; and C_O and C_C are the mean weights of the animals (except for the tumor) at the end of the experiment, in the experimental and control groups, respectively. A K_g with the sign (—) indicates a toxic effect of the substance on the organism [8].

In experiments on mice with L-1210 leukemia, antiblastic effect was evaluated from the ratio of mean duration of life (MDL) of the treated animals to the MDL of control animals, expressed in %. The numerical material was subjected to statistical treatment by the Fisher—Student method.

Before performance of therapeutic experiments, the toxicity of the substances tested was determined: the LD_{100} for mice on a one-time injection, and the maximum tolerable dose on daily injection for 6 days (MTD_6) for rats with Jensen sarcoma.

It was ascertained that the indicated 1-phenyl-3,3-dimethyltriazene derivatives are not very toxic: The MTD_6 for rats on administration *per os* in most of the compounds was 200 mg/kg or exceeded this figure. They give a considerable antiblastic effect, and have a large breadth of therapeutic action in experiments on rats with the Jensen sarcoma (Table 1). Here it was shown that the strength of tumor growth inhibition, the chemotherapeutic index, and also the antitumor spectrum of the substances studied, like their toxicity, depend on the character of the substituents in the para position of the benzene ring. Thus, in the degree of retardation of growth of the Jensen sarcoma in rats upon administration of equal

TABLE 1. Effect of 1-Phenyl-3,3-dimethyltriazene Derivatives on Growth of Jensen Sarcoma in Rats

Compound	Means of administration	Dose per time, mg/kg (number of treatments)	I_m %	K_g %	Compound	Means of administration	Dose per time, mg/kg (number of treatments)	I_m %	K_g %
I	p.o.	30 (7)	+85	+2			150 (7)	+84	+5
		40 (6)	+52	−1			200 (7)	+73	+4
II	p.o.	50 (5)	+35	0		p.o.	200 (5)	+82	−5
		100 (5)	+38	−5			50 (5)	+34	−3
		200 (5)	+54	−8	VII	p.o.	100 (5)	+33	−4
III	p.o.	50 (5)	+44	+1			200 (5)	+67	−4
		100 (5)	+51	−3			200 (5)	+80	−1
		200 (5)	+43	−9	VIII	p.o.	100 (6)	+76	+1
IV	p.o.	100 (6)	+30	−18	IX	p.o.	50 (7)	0	−7
		200 (5)	+89	−10			200 (5)	+83	−2
		25 (6)	+76	−2			50 (7)	+86	−7
		50 (6)	+75	−8	X	p.o.	100 (7)	+64	−8
		75 (6)	+67	−7			200 (5)	+76	−13
		100 (6)	+78	−6		i.p.	25 (6)	+71	+6
		200 (6)	+92	−17			50 (6)	+78	+1
V	i.p.	25 (7)	+28	+1			100 (4)	+84	−4
		50 (7)	+54	−3			200 (4)	+95	−22
		100 (7)	+92	+7			50 (7)	+59	−14
VI	i.p.	25 (7)	+75	+2	XI	p.o.	50 (7)	+59	−14
		50 (7)	+86	0			100 (6)	+55	+2
		100 (7)	+90	+1			200 (5)	+70	+1

Note: p.o.) *per os*; i.p.) intraperitoneally.

TABLE 2. Effect of 1-Phenyl-3,3-dimethyltriazene Derivatives on Development of L-1210 Leukemia, Line DBA/2, in Mice

Compound	Dose per time, mg/kg/day (no. of admin.)*	Increase in MDL of treated animals, %†
III	250 (6)	+44
IV	250 (4)	+56
VII	250 (6)	+81
IX	250 (4)	+32
X	250 (4)	+56
XI	250 (4)	0

*First injection on the day of inoculation of the animals; from then on, daily.

†The mean duration of life for mice in the control groups was 6.75-7.2 days.

doses *per os*, these substances may be divided into three groups. The least active proved to be ethers (II and III), which retarded tumor growth by 50% as a maximum. In compounds I, VII, and XI, the retardation index rose to 70-80%. The strongest antitumor effect (growth inhibition by 80-90%) on oral, or especially on intraperitoneal administration in doses of 100 or 200 mg/kg was given by derivatives containing an ester group in the para position of the benzene ring (IV-VI), and also amide groups mono- or disubstituted on the nitrogen atom (IX, X). Among the latter, compounds IV-VI had a high chemotherapeutic index (more than 10) on intraperitoneal injection, while in X it was only over 4. The derivatives IV and X differ from one another not only in their toxicity (the MTD_6 for rats on intraperitoneal injection is 200 and 100 mg/kg, respectively), but also in the spectrum of antitumor action. While compound IV on oral administration in doses of 100 or 200 mg/kg daily for 6 days retarded the growth of sarcoma 45 in rats by 30 and 70%, respectively, compound X, and also IX and XI, exerted no marked effect on the growth of this tumor.

Many of the substances of this type are active with respect of L-1210 leukemia in mice (Table 2). Thus, compounds IV, X, and IX, on daily administration *per os* in a dose of 250 mg/kg for 4 days extended the mean duration of life of animals with leukemia by 56 and 32%; and compounds VII and III, in the same doses, but with administration for six days, by 81 and 44%, respectively. In conclusion, one should note the good tolerance of these substances by the test animals (absence of side phenomena or sharp depression in weight gain).

Thus, 1-phenyl-3,3-dimethyltriazene derivatives which contain ether or ester, or amide groups which are mono- or disubstituted on the nitrogen atom in the para position of the benzene ring are not very toxic, and they exert a definite antiblastic action on Jensen sarcoma of rats or L-1210 mouse leukemia. The most active of these proved to be compounds IV-VI and X. In the case of compounds IV-VI, our data agree with the information available in the literature [4]. It is advisable to seek antitumor drugs among triazene derivatives, not only of the imidazole group and of benzoic acid, but also of other classes of chemical compounds.

EXPERIMENTAL

Structures of the compounds prepared were confirmed by elemental analysis. Purity of the compounds was checked by the use of thin-layer chromatography on Silufol UV-2254 plates in isopropyl alcohol. To develop the chromatograms we developed a convenient method of spot identification: spraying the plates with a solution of β-naphthol (0.05 g) in acetic acid (0.5 ml) and 10 ml of ethyl acetate. On cautious warming to 40-60°, the triazenes give an orange coloration.

1-(4-N,N-Dimethylbenzamido)-3,3-dimethyltriazene (X). To a solution of 4.0 g (0.024 mole) of p-amino-N,N-dimethylbenzamide in 8 ml of concentrated hydrochloric acid and 50 ml of finely chopped ice, with vigorous stirring and at a temperature of −5 to −2° was slowly added a 15% sodium nitrite solution (2.4 g in 12 ml of water), and the mixture was allowed to stand for 30 min. The cold solution of the diazo compound was added cautiously, with stirring, to a solution of 4.4 g (0.06 mole) of dimethylamine hydrochloride and 10 g of sodium bicarbonate in 100 ml of water, at 5-10°. The mixture was stirred for 2 to 3 h at room temperature. The precipitate which fell was filtered off, washed with water, and dried. Compound X (4.8 g, 89.5%) was obtained, m.p. 105-106° (from water), R_f = 0.44. Found, %: C 59.86; H 7.11; N 25.19. $C_{11}H_{16}N_4O$. Calculated, %: C 59.96; H 7.34; N 25.43.

Compound IX, 1-(4-N-methylbenzamido)-3,3-dimethyltriazene was prepared similarly in 99% yield, m.p. 144.5-145.5° (from benzene), R_f = 0.614. Found, %: C 58.25; H 6.75; N 27.23. $C_{10}H_{14}N_4O$. Calculated, %: C 58.23; H 6.83; N 27.15. 1-(4-Morpholinylbenzamido)-3,3-dimethyltriazene was prepared similarly, in 81% yield, m.p. 120-121° (from hexane), R_f = 0.484. Found, %: C 59.75; H 6.99; N 21.19. $C_{13}H_{18}N_4O_2$. Calculated, %: C 59.52; H 6.91; N 21.36.

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