SYNTHESIS AND ANTIMETASTATIC ACTIVITY OF 3,3-DIMETHYLTRIAZENE ARYL DERIVATIVES

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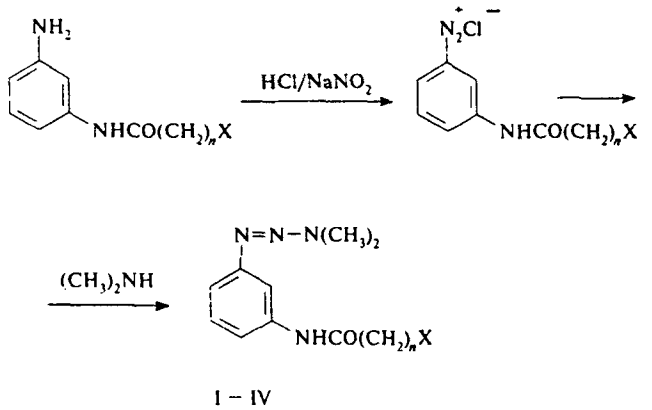
At present, practical oncology widely employs 5-(3,3-dimethyltriazeno)imidasole-4-carboxamide (DTIC) [1 – 3]. This compound is a highly effective antimetastatic agent, but also exhibits considerable toxicity because of rather low stability (especially with respect to photochemical decomposition with the formation of diazonium cation).

This circumstance explains the systematic interest of researchers toward investigation of the antimetastatic activity and mechanisms of action of 3,3-dimethyltriazene aryl derivatives [4 – 9]. It was found that some compounds of this class exhibit rather high stability. For example, potassium salt of 4-(3,3-dimethyltriazeno)benzoic acid (DM-COOK) is not subject to photochemical decomposition, while possessing the same antimetastatic activity as DTIC [6, 9 – 12].

It was established that a pharmocophore-carrier responsible for the antimetastatic activity of this series of compounds is represented by the 3,3-dimethyltriazene group, while electron-donor and electron-acceptor groups in the *para*- and *ortho*-positions only slightly affect the activity [5, 13, 14].

The purpose of this work was to study the synthesis and antimetastatic activity of the derivatives of 3,3-dimethyltriazenes with an acylated amino group in position 3 of the benzene ring (not influencing directly the triazene group).

N-[3-(3,3-Dimethyltriazeno)phenyl]halogenacylamides (I – IV) were obtained by azocombination of the corresponding phenyldiazonium salts with dimethylamine by the following scheme:

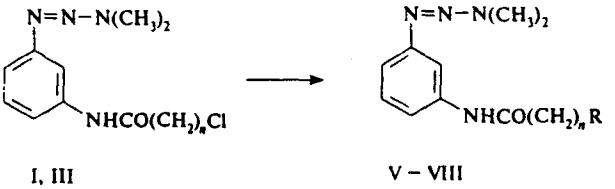


I – IV

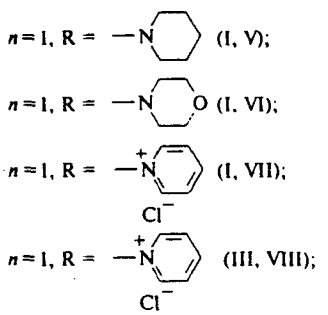
where $n = 1$, X = Cl (I); $n = 1$, X = Br (II); $n = 2$, X = Cl (III); $n = 2$, X = Br (IV).

The maximum yields were obtained for the combination of halogenacylaminophenyldiazonium salts with excess dimethylamine in the presence of sodium acetate.

The synthesized N-[3-(3,3-dimethyltriazeno)phenyl]-chloracetamide (I) and N-[3-(3,3-dimethyltriazeno)-phenyl]-β-chloropropionamide (III) were further modified by nucleophilic substitution of an amino group for the chlorine atom. The amines were represented by piperidine, morpholine, and pyridine; hydrogen chloride liberated during the reaction (for compounds V, VI) was bound by the excess amine.



I, III V – VIII



The structures of synthesized compounds were confirmed by ^{1}H NMR spectra (Table 1).

EXPERIMENTAL CHEMICAL PART

The ^{1}H NMR spectra were recorded on a Hitachi R-22 (Japan) spectrometer operated at a frequency of 90 MHz. The measurements were performed at 35°C in deuterochloroform (I, II, VI), deurteroacetone (III – V), deuteromethanol with HMDS internal standard (VII), and heavy water with DCS internal standard (VIII). Chemical shifts expressed in ppm (δ-scale).

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TABLE 1. Physicochemical Characteristics of Synthesized Compounds

Compound	Yield, %	M.p., °C	Empirical formula	^{1}H NMR spectrum, chemical shift δ
I	56	113 – 115	$C_{10}H_{13}N_4ClO$	3.25 (6H, bs, $2CH_3$), 4.09 (2H, s, CH_2), 7.13 – 7.37 (3H, m, H^4, H^5, H^6-arom.), 7.54 (1H, m, H_2-arom.), 8.29 (1H, bs, NH)
II	49	101 – 103	$C_{10}H_{13}N_4BrO$	3.26 (6H, bs, $2CH_3$), 3.85 (2H, s, CH_2), 7.05 – 7.40 (3H, m, H^4, H^5, H^6-arom.), 7.54 (1H, m, H^2-arom.), 8.35 (1H, bs, NH)
III	50	100 – 102	$C_{11}H_{15}N_4ClO$	2.81 (2H, t, CH_2CO), 3.28 (6H, bs, $2CH_3$), 3.86 (2H, s, CH_2Cl), 7.00 – 7.55 (3H, m, H^4, H^5, H^6-arom.), 7.80 (1H, m, H^2-arom.), 9.15 (1H, bs, NH)
IV	51	104 – 106	$C_{11}H_{15}N_4BrO$	2.97 (2H, t, CH_2CO), 3.27 (6H, bs, $2CH_3$), 3.72 (2H, s, CH_2Br), 7.00 – 7.55 (3H, m, H^4, H^5, H^6-arom.), 7.73 (1H, m, H^2-arom.), 9.16 (1H, bs, NH)
V	70	65 – 67	$C_{15}H_{23}N_5O$	1.20 – 1.80 (6H, m, $3CH_2$), 2.49 (4H, m, CH_2CH_2), 2.98 (2H, s, $COCH_2$), 3.28 (6H, bs, $2CH_3$), 7.00 – 7.40 (3H, m, H^4, H^5, H^6- arom.), 7.54 (1H, m, H^2-arom.), 9.29 (1H, bs, NH)
VI	68	125 – 127	$C_{14}H_{21}N_5O_2$	2.55 (4H, m, CH_2CH_2), 3.07 (2H, s, $COCH_2$), 3.27 (6H, s, $2CH_3$), 3.72 (4H, m, CH_2OCH_2), 7.5 – 7.45 (3H, m, H^4, H^5, H^6-arom.), 7.49 (1H, m, H^2-arom.), 9.03 (1H, bs, NH)
VII	51	182 – 184	$C_{15}H_{18}N_5ClO$	3.25 (6H, bs, $2CH_3$), 5.64 (2H, s, CH_2), 7.05 – 7.55 (3H, m, H^4, H^5, H^6-arom.), 7.64 (1H, m, H^2-arom.), 8.10, 8.62, 9.05 (5H, m, $H^{3,5}$, H^4, $H^{2,6}$-pyr. ring)
VIII	48	178 – 180	$C_{16}H_{20}N_5ClO$	3.20 (2H, m, CH_2CO), 3.35 (6H, bs, $2CH_3$), 5.00 (2H, m, CH_2), 7.06 – 7.58 (4H, m, C_6H_4), 8.11, 8.76, 9.11 (5H, m, $H^{3,5}$, H^4, $H^{2,6}$-pyr. ring)

Table 1 gives physicochemical characteristics of synthesized compounds. The results of elemental analysis agree with the calculated values.

N-[3-(3,3-dimethyltriazeno)phenyl]chloracetamide (I), N-[3-(3,3-dimethyltriazeno)phenyl]bromacetamide (II), N-[3-(3,3-dimethyltriazeno)phenyl]-β-chloropropion-amide (III), and N-[3-(3,3-dimethyltriazeno)phenyl]-β-bromopropionamide (IV). To a suspension of 0.05 mole of the corresponding N-(halogenacyl)-meta-phenylenediamine in 25 ml of water and 12.5 ml of concentrated hydrochloric acid at 0 – 2°C was added in drops a solution of 0.075 mole sodium nitrite in 25 ml of water. The mixture was stirred at the same temperature for 0.5 h and filtered. The filtrate was poured into a cooled (2 – 5°C) solution of 0.1 mole of dimethylamine hydrochloride, 0.1 mole KOH, and 0.5 mole sodium acetate in 500 ml water, and the reaction mixture was stirred for 3 h. Then the product was filtered, washed with water, dried, and recrystallized from benzene.

N-[3-(3,3-Dimethyltriazeno)phenyl]-2-*n*-piperidinacetamide (V) and N-[3-(3,3-dimethyltriazeno)phenyl]–morpholinacetamide (VI). To a solution of 0.005 mole I in 20 ml of benzene was added 0.01 mole of piperidine or mor-

TABLE 2. Toxicity and Antimetastatic-Antileukemic activity of 3,3-Dimethyltriazene Aryl Derivatives

Compound	Toxicity, mg/kg			Antimetastatic- antileukemic activity								
				STI, %			TGI, %			ALPI, %		
	LD_{100}	LD_{50}	MTD	L-1210	La	NK/Ly	EAT	WCS	S-45	L-1210	NK/Ly	EAT
I	300	250 (220 – 263)	100	109 $p < 0.01$	37	11		55	63 $p < 0.05$	100 $p < 0.01$	100, , $p < 0.01$	
II	> 25	23 (21 – 24)	20	85 $p < 0.05$	0	46	0	40	30	30	53 $p < 0.05$	100 $p < 0.05$
III	100	90 (87 – 93)	80	30	25	0	0	42		80 $p < 0.05$	100 $p < 0.01$	100 $p < 0.05$
IV	> 30	25 (23 – 26)	20	25	24	0	0	30	12	30	50	40
V	> 700	500 (495 – 530)	300	23	0	0	0	0		30	40	40
VI	> 900	700 (690 – 713)	400	25	30	12	30	40	15	35	41	45
VII	> 80	50 (47 – 56)	25	4	18	45	9	30	20	38	58 $p < 0.05$	100 $p < 0.05$
VIII	> 100	80 (77 – 87)	40	20	0	30	0	0		45	40	80 $p < 0.05$
DM-COOK		100 (82 – 128)	70	81 $p < 0.05$						100 $p < 0.01$		

pholine and the reaction mixture was boiled for 3 h. Then the residue was filtered and the solution evaporated. The remaining oily product was mixed with water, and the deposit was filtered and recrystallized from an ethanol : water (5 : 1) mixture with activated carbon.

3-(3,3-Dimethyltriazeno)phenylaminocarbonylmethylpyridinium chloride (VII) and 3-(3,3-dimethyltriazeno)phenylaminocarbonylethylpyridinium chloride (VIII). A solution of 0.005 mole of chloracetyl derivative I or β-chloropropionyl derivative III in 2 ml pyridine was heated at 90 – 100°C for 15 min, cooled, and diluted with ethyl ether. The residue was filtered and recrystallized from an ethanol : ethyl ether (1 : 3) mixture.

EXPERIMENTAL BIOLOGICAL PART

Acute toxicity of synthesized phenyltriazenes I – VIII was studied on 270 male ICR mice weighing 18 – 22 g. The acute toxicity parameters were determined according to the Litchfield – Wilcoxon method after a single intraperitoneal injection, and the statistical processing of data was performed using the Student's criterion [15].

The antimetastatic and antileukemic activities were assessed using the Walker carcinosarcoma (WCS), sarcoma 45 (S-45), Ehrlich ascites tumor (EAT), L-1210 lymphoid leukemia, NK/Ly ascites tumor, and La hemocytoblastoma. The compounds were intraperitoneally injected for 5 days, starting on the 3rd – 5th day after the tumor inoculation for the solid WCS and C-45 strains, and in 24 h for all other strains. The antimetastatic- antileukemic activity was judged by the relative (percentage) inhibition of tumor growth (TGI), survival time increase (STI), or inhibition of the ascite liquid production (ALPI) [16]. The antimetastatic activity was evaluated for 850 mice of the $C_{57}BL/C$, $C_{57}BL \times DBA$, and BAL B/C lines and 312 Wistar rats.

Comparative study of the effectiveness of test compounds and their close structural analog DM-COOK [6] was performed in parallel experiments with the L-1210 model.

The results of experiments (Table 2) showed that compounds II – IV, VII, and VIII are more toxic than I, V, and VI. Daily observations during a 30-day period showed normal exterior, behavior, and food consumption of animals upon the maximum tolerated drug administration.

Compound I exhibited the maximum antiblastic action among the substances studied: the growth of WCS and S-45 tumors was inhibited by 55 and 63%, respectively, and the lifetime of L-1210 La-injected animals increased by 109 and 37%, respectively. Compound I also inhibited the production of L-1210 ascite liquid and reduced the spleen mass by up to 40% for the La injections. With respect to L-1210, the antimetastatic activity of compound I was higher than that of DM-COOK. The bromacetyl analog II also exhibited a pronounced antileukemic activity (STI,85%), while compounds III, IV, and VI showed only a tendency to increase the lifetime. Phenyl-3,3-dimethyltriazenes V, VII, and VII did not affect the lifetime. Note that all studied compounds reduced or hindered the production of ascite liquid upon the L-1210, NK / Ly, and EAT injections.

Blood analyses showed that compound I reduced the number of leukocytes by 30 – 50% ($p < 0.01$) against the untreated control, and compound VII (with respect to L-1210) by 54% ($p < 0.05$). The other compounds did not significantly affect the number of leukocytes for the experimental tumors.

In conclusion, the highest antiblastic effect among the compounds studied was observed for aryltriazenes containing halogenacylated amino group (I – III) and a triazene derivative of aminocarbonylmethylpyridinium chloride (VII).

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