SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 3-SUBSTITUTED-5H-4-OXO-1,2,3-TRIAZINO[5,4-b]INDOLES AND 1,1-DIALKYL(1-ARYL)-3-(2-ETHOXYCARBONYLINDOL-3-YL)TRIAZENES

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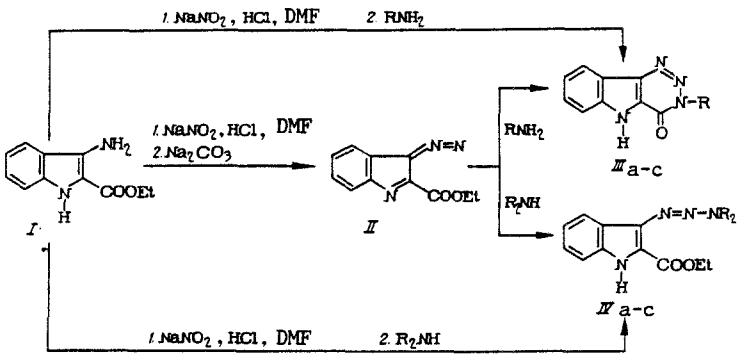
UDC 615.227.3:547.336.2].012.1

Among polyazahetero[b]indoles in which the heterocyclic ring is connected with position 3 of the indole by a nitrogen atom in the ring are found many compounds that have antiviral, antitumor, and antiinflammatory activities [7, 10, 11]. Among them are 1,2,4-triazino[5,6-b]indoles [3-6], and also 1,2,3-triazino- and pyrimido[5,4-b]indoles have come within reach recently [9]. The same kinds of activity are also found in some indoles with a nitrogen atom at the 3-position: 1,1-dialkyl-3-(2-ethoxycarbonylindol-3-yl)triazenes, 3H-3-diazo-2-ethoxycarbonylindoles, and N-(indol-3-yl)-N^1,N^1-dialkylamides [2, 8].

The aim of this work is the synthesis and investigation of the antiviral and antibacterial activities of 5H-1,2,3-triazino[5,4-b]indoles (IIIa-c) and 2-ethoxycarbonyl-3-indolyltriazenes (IVa-c) with different substituents at one of the terminal nitrogen atoms.

Triazinoindoles IIIa-c were prepared by a known method [9], which consists of reaction of 2-ethoxycarbonyl-3H-diazoindole (II) with ammonia, methylamine, and benzylamine. The yields of triazinoindoles IIIa-c was 25-61%. Starting diazoindole II was prepared by successive treatment of the hydrochloride of amine (I) in water with a diazotizing mixture and sodium bicarbonate.



III: R = a) R=H, b) Me, c) CH_2Ph

IV: R = a) Et, b) NR_2 - morpholino, c) N-phenylpiperidino

In order to increase the yield of desired product and to simplify the method, we have carried out the step of diazotization of amine I in a 1.6:1 mixture of DMF and water, diazoindole II was not isolated, and combination of the diazo solution with the amine component was carried out at pH 9, which parameter was maintained with an excess of reagent.

These changes led to an increase in the yield of triazinoindoles IIIa-c to 61-86%. By the same method, using the secondary amines diethylamine, morpholine, and N-phenylpiperazine, triazenes IVa-c were obtained in yields of 61-83%. Obviously, providing pH 9 of the medium with an excess of the amine component deprotonates the diazonium salt initially formed from amine I and also converts it to diazoindole II. It was shown earlier that already at pH 7 the diazoindole is isolated from an aqueous diazo solution in a yield of 73% [9]. Then diazoindole II reacts with the amine component and is converted to the corresponding triazene and in the cases of ammonia and primary amines the latter cyclizes to the triazino-

S. Ordzhonikidze All-Union Scientific-Research Institute of Pharmaceutical Chemistry, Moscow. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 24, No. 11, pp. 36-40, November, 1990. Original article submitted November 28, 1989.

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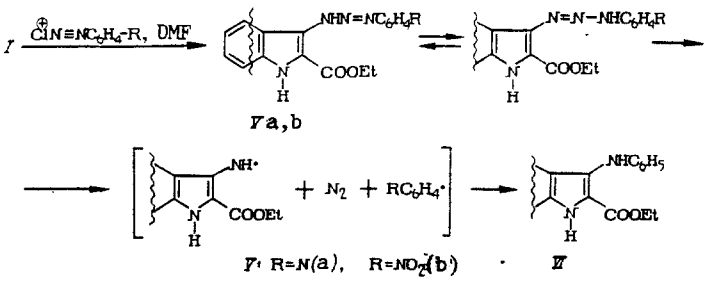
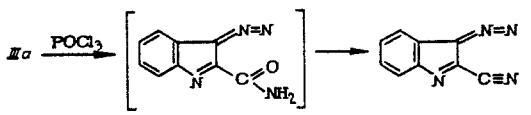


indole. A different result was found when using aniline as amine; the only product proved to be 3-phenylaminoindole (VI), which was isolated in a yield of 14%. In the PMR spectrum of amine VI in DMSO-d_6 are found, in addition to the signals of the aromatic protons and of the ethoxycarbonyl substituent, singlet signals of the protons of the NH groups of indole and 3-phenylamino at 11.38 and 7.98 ppm, respectively.

For the preparation of 3-indolyltriazenes with aryl substituents at the terminal nitrogen atom of the triazene chain we used one more method of approach, combination of amine I with an aryldiazonium salt. The diazo constituents used were aniline and p-nitroaniline, and the reaction was carried out in a 1.6:1 mixture of DMF and water at pH 7 and 0°C.

Triazene Va was isolated in a yield of 10%. According to PMR spectral data recorded in DMSO-d_6, triazene Va is present as a mixture of tautomers in the ratio of 1:1, with which correspond two sets of signals of the NH groups of the indole and triazene substituents at 11.81 and 12.24, and 8.23 and 8.19 ppm, respectively. Reaction of amine I with p-nitrophenyldiazonium salt yielded a mixture of triazene Vb and starting amine I. The composition of the mixture was determined by data from TLC and the mass spectrum, in which, in addition to a peak of the molecular ion M^+ 203, corresponding with the mass of amine I, a molecular ion peak M^+ 353, corresponding with the mass of triazene Vb, is present. In contrast to 1,1-dialkyltriazenes IVa-c, 1-aryltriazenes Va, b are thermally unstable compounds. Thus, triazene Va is converted to secondary amine VI when heated in DMF at 60°C for 1 min and formation of 5H-4-oxo-3-phenyl-1,2,3-triazino-[5,4-b]indole is not observed.



Obviously, the spontaneous decomposition of intermediate triazene Va is the cause of the formation of amine VI from diazoindole II and aniline.

We suppose that the decomposition of triazene Va proceeds in the same way as the thermal decomposition of phenyl-substituted triazenes, which proceeds by a radical mechanism and is accompanied by the formation of benzene, biphenyl, and aniline [12]. On treatment of triazinoindole IIIa with phosphorus oxychloride there takes place a reaction that is the reverse of the cyclization with simultaneous conversion of the amide group to the nitrile group, with the result that diazoindole VII is formed.

The IR spectrum of diazoindole VII contains absorption bands of the C≡N and N=N groups at 2240 and 2110 cm^{-1}, respectively. The mass spectrum of diazoindole VII contains an intensive peak of the molecular ion M^+ 168. To the presence of diazo and cyano groups point the appearances of ions with m/z 140 $[M - N]^+$, 142 $[M - CN]^+$, and 113 $[M - N_2{-}CN]^+$.

EXPERIMENTAL (CHEMICAL)

IR spectra were taken on a Perkin–Elmer 599 spectrometer (Sweden) from suspensions in paraffin oil. PMR spectra were recorded on a Varian XL-200 spectrometer with TMS as internal standard. The progress of the reaction and the purity of the prepared products were checked by TLC on Silufol UV-254 plates in the systems benzene–acetone 9:1 and $CHCl_3$, and spots were visualized with UV light.

Characteristics of compounds IIIa-c, IVa-c, and V-VII are summarized in Table 1. Found and calculated values of elemental analyses were in agreement.

5H-4-Oxo-1,2,3-triazino[5,4-b]indoles (IIIa-c). A. To a solution of 5 mmoles of diazoindole II [9] in 20 ml of ethanol is added 10 mmoles of the appropriate amine. The reaction

TABLE 1. Characteristics of Compounds III-VII

Compound	Yield, %	Mp, °C (solvent)	Empirical formula	IR spectrum ν_{max}, cm^{-1}	
				C=O	NH
IIIa*	86	215** (50 % alcohol)	$C_9H_6N_4O$	1670	3150, 3440
IIIb*	66	236** (DMF–dioxane, 1:1)	$C_{10}H_8N_4O$	1670	3130
IIIc*	61	275** (75 % DMF)	$C_{16}H_{12}N_4O$	1670	3140
IVa	61	156–158 (75 % alcohol)	$C_{15}H_{20}N_4O_2$		
IVb	83	140–141 (50 % alcohol)	$C_{15}H_{19}N_4O_3$	1670	3300
IVc	76	156–158 (50 % alcohol)	$C_{21}H_{23}N_5O_2$	1670	3300
Va	10	141–142** (petroleum ether–benzene 1:1)	$C_{17}H_{16}N_4O_2$	1680	3350, 3100
VI	14	166–167 (petroleum ether–benzene 1:1)	$C_{17}H_{16}N_2O_2$	1670	3310, 3410
VII***	25	120–122 (petroleum ether–ethyl acetate 9:1)	$C_9H_4N_4$		

*Yields of IIIa-c by method A in %: a) 36; b) 50; c) 61.
**The compounds melt with decomposition.
***IR spectrum, ν_{max}, cm^{-1}: 2240 (CN), 2110 (N=N).

TABLE 2. Antimicrobial Activities of Triazino[5,4-b]indoles IIIa-c and Triazenes IVa-c

Compound	MIC, μg/ml							
	St. aureus 209-P	Bac. subtilis 6633 ATCC	E. coli 25922 ATCC	Prot. vulgaris 6896 ATCC	Ps. aeruginosa 165	M. canis 3/84	Tr. gypseum 5/85	C. albicans 1755
IIIa	125	>250	>250	>250	>250	>250	>250	>250
IIIb	>250	>250	>250	>250	>250	>250	>250	>250
IIIc	>250	>250	>250	>250	>250	>250	>250	>250
IVa	0,25 / MBC* 0,5	0,015	15,6	15,6	>250	7,8	7,8	3,9
IVb	0,5 / MBC 1,0	1,0	15,6	31,2	>250	7,8	7,8	2,0
IVc	1,0 / MBC 2,0	15,6	>250	>250	>250	15,6	15,6	7,8

*Minimum bactericidal concentration.

mixture is heated to 60-65°C, allowed to stand for 3-5 min, cooled to 22°C, and allowed to stand for 3 h. The excess of amine is neutralized with 0.1 N HCl to pH 7, the precipitate is filtered off, and crystallized from 50% alcohol.

B. To a solution of 7.3 mmoles of amine I [1] in a mixture of 25 ml of DMF and 15 ml of water is added at 0-5°C with stirring 1.3 ml (≈10 mmoles) of concentrated HCl, the mixture is cooled to 0-2°C, at that temperature a solution of 0.58 g (8.3 mmoles) of $NaNO_2$ in 10 ml of water is added, the mixture is stirred at 5°C for 0.5 h, 16 mmoles of the appropriate amine is added (the pH of the solution is 8-9), and then the reaction mixture is heated to 60-65°C for 1 min. The mixture is cooled to 20°C and kept at that temperature for 3 h. The excess of amine is neutralized with 0.1 N HCl to pH 7. The precipitate is filtered off.

1,1-Dialkyl-(1-aryl)-3-(ethoxycarbonylindol-3-yl)triazenes (IVa-c). To a diazo solution prepared from 1.5 g (7.3 mmoles) of I, 25 ml of DMF, 15 ml of water, 1.32 ml of concentrated HCl, and 0.58 g (8.3 mmoles) of $NaNO_2$, is added at 5°C an excess of the appropriate amine to pH 8-9, the reaction mixture is heated to 60-65°C for 1-3 min, cooled, the precipitate is filtered off, washed with water, dried, and crystallized from the appropriate solvent.

1-Phenyl- and 1-(p-Nitrophenyl)-3-(2-ethoxycarbonylinol-3-yl)triazenes (Va, b). To a solution of 1 g (5 mmoles) of amine I in 16.6 ml of DMF and 10 ml of water is added at 0°C a diazo solution prepared from 5 mmoles of aniline or p-nitroaniline, 0.35 g (5 mmoles) of $NaNO_2$, and 1.1 ml of concentrated HCl, and the mixture is neutralized to pH 3 with sodium acetate. During the addition of the diazonium salt solution the pH of the reaction mixture is kept within 7-7.5 by addition of a saturated solution of Na_2CO_3. The reaction mixture is stirred at 0°C for 0.5 h, the precipitate is filtered off, and dissolved in 5-10 ml of benzene. The insoluble fraction (starting amine I) is filtered off, petroleum ether is added to the mother liquor, the precipitate is filtered off and washed with petroleum ether. Yield 0.15 g of Va. PMR spectrum of Va (DMSO-d_6), δ, ppm: 1.38 t (CH_3), 4.42 q (CH_2), 7.05-7.54 (aromatic protons), 8.19 s, 8.23 s (NH of triazene), 11.81, 12.24 s (NH of indole). The yield of Vb is 0.25 g in a mixture with starting amine I. M^+ 203, M^+ 353.

2-Ethoxycarbonyl-3-phenylaminoindole (IV). A. To a diazo solution prepared from 0.5 g (2.5 mmoles) of amine I, 8 ml of DMF, 5 ml of water, 0.44 ml of concentrated HCl, and 0.19 g (2.6 mmoles) of $NaNO_2$ is added 1 ml (12 mmoles) of aniline, the mixture is heated at 60°C for 1 min, cooled to 20°C, the oil that is formed is separated off, dissolved in $CHCl_3$, dried with $CaCl_2$, and chromatographed over a silica gel column using $CHCl_3$ as eluent. The fraction with R_f 0.65 ($CHCl_3$) is collected, and the solvent is evaporated to yield 0.08 g of VI.

B. In a mixture of 5 ml of DMF and 3 ml of water is dissolved 0.3 g (1 mmole) of triazene V, the solution is heated at 60°C for 1 min and cooled to 20°C. The solvent is evaporated under vacuum and the residue is crystallized from a 1:1 mixture of petroleum ether and benzene to yield 0.03 g of VI. PMR spectrum (DMSO-d_6), δ, ppm: 1.26 t (CH_3), 4.28 q (CH_2), 6.99-7.73 m (aromatic protons), 7.98 s (NH of triazene), 11.38 s (NH).

2-Cyano-3H-diazoindole (VII). A mixture of 0.7 g (3.7 mmoles) of triazinoindole IIIa, 0.7 ml of Et_3N, and 5 ml of $POCl_3$ is stirred at 70°C for 7 h. The reaction mixture is poured out on 50 ml of an ice–water mixture and alkalized to pH 7-8 with Na_2CO_3. The precipitate is filtered off, washed with water, and dried. The residue is dissolved in $CHCl_3$ and chromatographed over a silica gel column with $CHCl_3$ as eluent. The fraction with R_f 0.37 ($CHCl_3$) is collected, the solvent is evaporated, and the residue is crystallized from a petroleum ether–ethyl acetate mixture to yield 0.15 g of VII.

EXPERIMENTAL (BIOLOGICAL)

The antiviral activity of compounds IIIa-c and IVa-c were studied with regard to virus group A, strain A/FPV(H7N7).

It was found that the compounds studied did not have virus-inhibiting activity on the virus group.

The antimicrobial activity of compounds IIIa-c and IVa-c was studied in vitro by the method of twofold serial dilution in a liquid culture medium. In experiments with bacteria we used Hottinger's broth and in experiments with fungi Sabouraud agar. The microbial charge was $1 \cdot 10^6$ pfu/ml for bacteria and $1 \cdot 10^6$ pfu/ml for fungi. The compounds were tested in concentrations of 250 μg/ml and lower. The antibacterial activity was considered after 18-20 h of incubation of the inoculation in a thermostat at 37°C and the activity with regard to yeast-like fungi of the species C. albicans 1755 was judged after 1 day in a thermostat at 25°C; the activity with regard to inducers of skin dermatomycoses (M. canis 3/84 and Tr. gypseum 5/85) was judged after 5 days under the same conditions of cultivation (25°C). In experiments with animals we determined the maximum transferable dose of the compound at 5-days intragastric and intraperitoneal administration. The model of encephalomeningitis in mice was produced by intracerebral infection with C. albicans 1755 at a dose of $4 \cdot 10^7$ pfu. Treatment was started immediately after infection and conducted for 5 days. The results of the tests were judged 10 and 30 days after the infection. Microsporia in guinea pig was evoked by application and rubbing-in of the pathogen M. canis 3/84 on epilated and scarified parts of the skin of the back of the animals. The compounds were studied by local application as 1% ointments. Treatment was started 8 days after the infection and lasted 3 weeks. The results were judged 1 and 7 days after the last day of treatment.

It is clear from Table 2 that compounds IVa-c have high activity against staphylococci. The minimum inhibitory concentrations (MIC) are 0.25, 0.5, and 1.0 μg/ml, respectively. The compounds possess a high bactericidal effect in concentrations of 0.5, 1.0, and 2.0 μg/ml, respectively. Compounds IVa, b are moderately active with regard to Escherichia coli and Proteus vulgaris (MIC 15.6-31.2 μg/ml). Compound IVb is highly active, and compounds IVa and

IVc are active against C. albicans (MIC 2.0 and 3.9-7.8 μg/ml). Compounds IVa-c suppress the growth of skin dermatophytes in concentrations of 7.8-15.6 μg/ml. All compounds studied are inactive against Pseudomonas aeruginosa, and compounds IIIa-c do not have antimicrobial activity in concentrations of 250 μg/ml. The maximum transferable dose for mice in case of 5-day intragastric and intraperitoneal administration was 62.5 mg/kg for compound IVb and 125 mg/kg for compound IVc. According to the model of candidosis encephalomeningitis in mice compound IVc, in case of intragastric administration at a dose of 31.2 mg/kg, shows a weak therapeutic effect on the 10th day of observation: the total life span of the animals relative to the maximally possible was 79%, and in the control group 65%. On the 30th day of the experiment the total life span of the animals was 35% and in the control group 25%. Compound IVb did not show activity. In the same system of application the control preparation mycoheptin at a dose of 80 mg/kg gave a total life span of the mice of 100% on the 10th day and 98% on the 30th day. With the model of microsporia of guinea pigs compound IVb, in the case of local application as a 1% ointment, did not show therapeutic activity with regard to the pathogen of microsporia.

Thus, triazenes derivatives IVa-c have a broad spectrum of antimicrobial activity in vitro: they have high bacteriostatic and bactericidal effects with regard to staphylococcus, a moderate activity against Gram-negative bacteria (except compound IVc), and they are active against pathogenic fungi. Compound IVc shows weak therapeutic activity in the case of candidosis encephalomeningitis in mice. However, in the latest experiments with the model of acute bacterial infections of mice in case of intragastric infection, these compounds did not show chemotherapeutic effectivity (data from L. D. Shchipilova). Triazino[5,4-b]indole derivatives IIIa-c do not possess antibacterial and antifungal activities in vitro.

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