ANTIMICROBIAL ACTIVITY OF NITRO DERIVATIVES OF AZOLO[1,5-a]-PYRIMIDINE AND AZOLO[5,1-c][1,2,4]TRIAZINE

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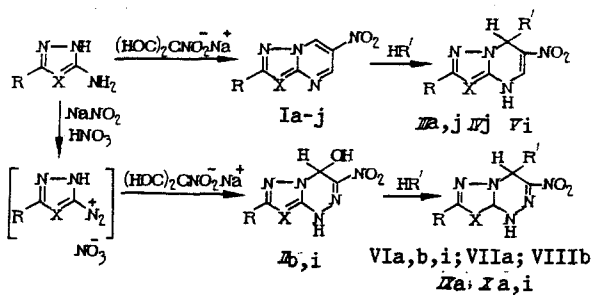
Derivatives of 6-nitro-5,6-dihydrouracil inhibit the growth of Gram-positive and Gram-negative bacteria [8]. There was observed antimicrobial activity of azolo-condensed derivatives of pyrimidine and 1,2,4-triazine: imidazo[1,2-a]pyrimidine and triazolo[3,4-c][1,2,4]triazine [6, 7]. These data point to promise of the search for compounds with antimicrobial activity in the series of nitro derivatives of azoloazines with a nitrogen atom at a bridge position (I-X).

To search for effective antimicrobial preparations and also to study the relationship between their structure (nature and position of the substituents at the azole and azine parts of the molecule) and the biological activity we have carried out the synthesis of a series of nitro derivatives of azolo[1,5-a]-pyrimidine (I, III, IV, V) and also of their aza analogs, azolo[5,1-c][1,2,4]triazines (II, VI-X) (see reaction scheme).

6-Nitro-1,2,4-triazolo[1,5-a]pyrimidines (Ia-h) and 6-nitropyrazolo[1,5-a]pyrimidines (Ii-j) were prepared according to [3] by condensation of 3-R-5-amino-1,2,4-triazoles and 4-R-5-aminopyrazoles with the sodium salt of nitromalonic dialdehyde. The increased π-deficiency of nitropyrimidines I results in the fact that these compounds easily add to nucleophiles with formation of σ-adducts. Thus, on reaction with indoles or resorcinol according to [2, 5] are obtained 7-(indolyl-3') or 7-(2,4-dihydroxyphenyl) derivatives of 6-nitro-4,7-dihydroazolo[1,5-a]pyrimidines (III-V).



I—X: *a*) R=H, X=N; b) R=Me, X=N; c) R=SMe, X=N; d) R=SEt, X=N; e) R=SPr, X=N; f) R=NH_2, X=N; g) R=CF_3, X=N; h) R=Ph, X=N; i) R=H, X=CCOOEt; j) R=H, X=CNO_2; III, VI:R'=indolyl-3, IV, VII:R'=2-methyl-indolyl-3 V:R'=2,4-dihydroxyphenyl VIII:R'=OEt IX:R'=OPr X:R'=OBu

Nitro derivatives of azolo [5,1-c][1,2,4]triazine (IIb, i) were synthesized according to [4] by reacting the diazonium salt, prepared from the corresponding aminoazoles, with the sodium salt of nitromalonic dialdehyde. The reaction includes the formation of azolylhydrazones of the nitroglyoxals (similar to the Japp—Klingemann reaction), which easily cyclize to azole annelated nitrotriazines II. When compounds II are heated with alcohols, substitution of the hydroxy groups with alkoxy groups takes place and alkoxytriazines VIII-X are formed. We have found that compounds II react just as their deaza analogs I with indoles, which leads to the preparation of 7-indolyl-4,7-dihydroazolo[5,1-c][1,2,4]triazines (VI-VII).

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TABLE 1. Physical Constants of 6-Nitroazolo[1,5-a]pyrimidines and 6-nitroazolo[5,1-c][1,2,4]triazines

Compound	Yield, %	mp, °C	Empirical formula	IR spectra, ν_{max}, cm^{-1}	PMR spectra (DMSO-d_6), δ, ppm
Id	80	112—115	$C_7H_7N_5O_2$	1350, 1560 (NO_2)	1,30(3H,t,CH_3), 3,20(2H,q,CH_2), 950(1H,d,7-H), 10,52(1H,d,5-H)
Ie	76	110—111	$C_8H_9N_5O_2$	1355, 1560 (NO_2)	1,20(3H,t,CH_3), 1,80(2H,sextet,CH_2), 3,27(2H,t,CH_2), 9,51(1H,d,7-H), 10,46(1H,d,5-H)
Ig	70	140—141	$C_6H_2N_5O_2F_3$	1360, 1580 (NO_2),	9,75(1H,d,7-H), 10,83(1H,d,5-H)
VIa	62	283—285	$C_{12}H_9N_7O_2$	1330, 1570 (NO_2), 3150 (NH), 3310 (NH)	7,32(1H,s,7-H), 7,66(1H,d,2′-H) 7,60—7,00(5H,complex m, 4′-7′-H,NH′), 7,86(1H,s,2-H), 11,45(1H,br.s,NH)
VIb	60	253—255	$C_{13}H_{11}N_7O_2$	1325, 1545 (NO_2), 3200—3270 (NH)	2,20(3H,s,CH_3), 7,19(1H.s7-H) 7,53—6,98(5H, complex m, 4′-7′-H,NH′), 7,58(1H,d,2′-H), 11,39(1H,br.s,NH)
VIi	51	295—298	$C_{16}H_{14}N_6O_4$	1330, 1590 (NO_2), 1685 (CO), 3300 (NH)	1,30(3H,t,CH_3), 4,30(2H,q,CH_2), 6,90(1H,s,7-H), 7,40—6,90(5H,m,4′-7′-H, NH′, 7,75(1H,s,2-H); 11,30(1H,br.s,NH), 12,35 (1H,br.sNH)
VIIa	49	293—295	$C_{13}H_{11}N_7O_3$	1395, 1542 (NO_2), 3210—3355 (NH)	2,58(3H,s,CH_3), 7,27(1H,s,7-H) 7,50—6,90(5H, sextet ,m,4-7-H,NH′), 7,80(1H,s,2-H), 11,33(1H,br.s,NH)
IXa	70	214—215	$C_7H_{10}N_6O_3$	1344, 1550 (NO_2), 3210 (NH)	1,10(3H,t,CH_3),3,65(2H,sextet,CH_2),3,90(2H,t, CH_2), 7,22(1H,s,7-H), 8,42(1H,s,2-H)
Xa	64	194—196	$C_8H_{12}N_6O_3$	1345, 1540 (NO_2), 3150 (NH)	1,10(3H,t,CH_3), 3,35—4,00(6H, complex m,O-CH_2), CH_2, CH_2), 7,20(1H,s,7-H), 8,40(1H s,2-H)
Xi	68	128—130	$C_{12}H_{17}N_5O_5$	1345, 1545 (NO_2), 1700 (CO), 3200 (NH)	0,60—1,10(4H,m,CH_2,CH_2), 1,30(6H,br. t, CH_3, CH_3), 3,78(2H,br.t,CH_2), 4,30(2H,q,O-CH_2), 6,87(1H,s,7-H), 8,07(1H,s,2-H), 12,70(1H,br.s,NH)

The structures of the prepared compounds were confirmed by IR, UV, and PMR spectral data. The physicochemical characteristics of the novel compounds are listed in Table 1. Bands of the valence vibrations of the nitro group are present in the IR spectra of all the prepared compounds. PMR spectral data completely correspond with the assigned structures.

EXPERIMENTAL (CHEMICAL)

IR spectra were taken in paraffin oil on a UR-20 spectrometer (GDR). PMR spectra were taken from DMSO-d_6 solutions on a Bruker WH-90 spectrometer (FRG) (80 MHz, internal standard HMDS). Found and calculated values of elemental analyses matched.

2-R-6-Nitroazolo[1,5-a]pyrimidines (Ia-j). A solution of 0.01 mole of the appropriate aminoazole in 15 ml of 2 N HCl is mixed with a solution of 1.6 g (0.01 mole) of the sodium salt of nitromalonic dialdehyde and the mixture is stirred at 20-25°C for 30 min. The precipitate is filtered off, crystallized from alcohol, and dried under vacuum over P_2O_5 at 100-150°C. The main characteristics of compounds Ia-c, f, h, i correspond with those described in [3] and those of compounds Id, e, g are listed in Table 1.

2-R-6-Nitroazolo[5,1-c][1,2,4]triazines (IIb, i). To a solution of 0.01 mole of the corresponding aminoazole in 1.6 ml of HNO_3 (d = 1.4) and 10 ml of water, cooled to 0°C, is added over 15 min a solution of 0.8 g (0.11 mole) of $NaNO_2$ in 5 ml of water. The mixture is kept at that temperature for 10 min, then mixed with a solution of 1.4 g (0.01 mole) of the monohydrate of the sodium salt of nitromalonic dialdehyde in 5 ml of water, and stirred at 20°C for 2 h. The precipitate is filtered off, crystallized from water, and dried at 100°C over P_2O_5 under vacuum. The main characteristics of compounds IIb, i correspond with those described in [4].

2-R-6-Nitro-7-alkoxy-4,7-dihydroazolo[5,1-c][1,2,4]triazines (VIIIb, IXa, Xa, i). A solution of 0.01 mole of the nitroazoloazine (IIa, b, i) in the appropriate alcohol is refluxed for 30 min and filtered while still hot. The precipitate that is formed on cooling is filtered off and dried under vacuum over P_2O_5 (at the boiling temperature of the solvent). The main characteristics of compound VIIIb correspond with those described in [4] and those of compounds IXa and Xa, i are listed in Table 1.

2-R-6-Nitro-7-(indolyl-3')-4,7-dihydroazolo[1,5-a]pyrimidines (IIIa, j, IVj) and 6-nitro-7-(indolyl-3)-4,7-dihydroazolo[5,1-c][1,2,4]triazines (VIa, b, i, VIIa). A mixture of 0.01 mole of the corresponding nitroazoloazine and 0.01 mole of indole is refluxed in butanol for 30 min. The precipitate is filtered off, washed liberally with ether, crystallized from butanol, and dried at 100°C over P_2O_5 under vacuum. The main characteristics of compounds IIIa, j and IVj correspond with those described in [2] and those of compounds VIa, b, i and VIIa are listed in Table 1.

3-Ethoxycarbonyl-6-nitro-7-(2,4-dihydroxyphenyl)-4,7-dihydroazolo[1,5-a]pyrimidine (Vi). A mixture of 0.01 mole of the nitroazoloazine and 0.02 mole of resorcinol is heated in ethanol at 70°C for 25-30 min. The precipitate that is formed on cooling is filtered off, washed with ether, crystallized from ethanol, and dried under vacuum over P_2O_5 at 80°C [5].

EXPERIMENTAL (BIOLOGICAL)

The antimicrobial activities of the synthesized compounds were determined with the serial dilution method [1] with regard to Gram-positive and Gram-negative conditionally pathogenic microorganisms, yeast-like and phytopathogenic fungi, and dermatophytes.

The strains *Staphylococcus aureaus* 209, *Staphylococcus albus*, *Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* were grown on agar and beef-extract broth at 37°C for 18-24 h. The strain *Corynebacterium divericatum* was grown under the same conditions at 28°C. The strains of the yeast-like fungi *Candida albicans*, *Candida crusei*, and *Candida tropicalis* were grown at 37°C for 18-24 h on agarized and liquid must. The strains of the phytopathogenic fungi *Fusarium oxysporum*, *Aspergillus niger*, and *Verticillium dahliae* were grown in the same medium and the dermatophytes *Epidermophyton flociasum*, *Trichophyton griseum*, and *Microsporum lanosum* at 28°C for 72-168 h. The microbial load was 200,000 microbial cells per ml medium. The degree of antimicrobial activity of the compounds was judged by the value of the minimal concentration that inhibits the growth of the bacteria (MIC, μg/ml).

It was found that the antimicrobial activity of these compounds slightly depends on the substituent on the azole part of the molecule and, as a rule, is related to the nature of the substituent at the 7-position. Nitroazolopyrimidines Ia-j, which are not substituted at this position, are mainly active against fungi and at the same time possess weak antistaphylococcal activity. MIC of the most active compounds Id and Ie, against microorganisms resistant to the majority of chemotherapeutic preparations and dermatophytes, was 10-20 μg/ml for yeast-like fungi of the genus *Candida*.

Introduction of a substituent at the 7-position (IIIa, j; IVj; Vi), and at the same time using the 2-aza analogs of nitro derivatives of 1,2,4-triazine (IIb, i; VIa, b, i; VIIa; VIIIb; IXa; Xa, i), changes the spectrum of antimicrobial activity. These compounds inhibit the growth of Gram-positive microorganisms and have considerable influence on the degree of antimicrobial activity. Introduction of a resorcinol, indole, or alcohol radical increases the antistaphylococcal activity to 20-50 μg/ml (Vi; VIb, i; IXa; Xa). Altogether, the investigated series of compounds shows the largest antimicrobial activity against Gram-positive and Gram-negative conditionally pathogenic microorganisms (*Bacillus cereus*, *Corynebacterium divercatum)* and the yeast-like fungus *Candida albicans*. Of the 23 compounds investigated 48% have an activity to 50 μg/ml.

LITERATURE CITED

1. N. S. Egorov, Fundamentals of the Studies of Antibiotics [in Russian], 3rd edn., Moscow (1979), pp. 46-47.
2. T. L. Pilicheva, V. L. Rusinov, O. N. Chupakhin, et al., Khim. Geterotsikl. Soed., No. 11, 1544-1549 (1986).
3. V. L. Rusinov, I. Ya. Postovskii, A. Yu. Petrov, et al., Khim. Geterotsikl. Soed., No. 11, 1554-1556 (1981).
4. V. L. Rusinov, T. L. Pilicheva, O. N. Chupakhin, et al., Khim. Geterotsikl. Soed., No. 5, 662-665 (1986).
5. V. L. Rusinov, T. L. Pilicheva, A. V. Myasnikov, et al., Khim. Geterotsikl. Soed., No. 8, 1137-1138 (1986).
6. C. Cristescu, Romanian Patent 55848, Ref. Zh., Khim., No. 60116P (1975).
7. C. Cristescu, Romanian Patent 55848, Ref. Zh., Khim., No. 210187P (1975).
8. R. A. Long, T. R. Matthews and R. K. Roland, J. Med. Chem., **19**, 1072 (1976).