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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 5-(ARYLMETHYLIDENE)-2,4,6-PYRIMIDINE-2,4,6(1*H*,3*H*,5*H*)-TRIONES

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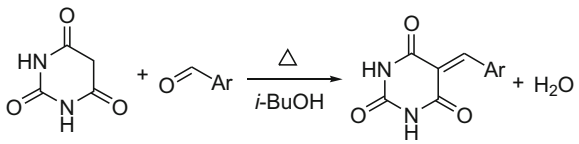
A series of 5-(arylmethylidene)-2,4,6-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones were synthesized and tested for antimicrobial activity against *Staphylococcus* and *Streptococcus* bacteria strains.

Keywords: synthesis, 5-(arylmethylidene)-2,4,6-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones, antimicrobial activity, minimum inhibitory and bactericidal concentrations.

The antimycobacterial activity of a series of 5-(arylmethylidene)-2,4,6-pyrimidine-2,4,6(1*H*,3*H*,5*H*)triones against *M. lufu* and their acute daily toxicity were previously studied by us [1].

In continuation of research in this area and to study the spectrum of biological activity, we improved the method for preparing 5-(arylmethylidene)-2,4,6-pyrimidine-2,4,6(1*H*,-3*H*,5*H*)-triones (**VIII**-**XIII**) and studied their antimicrobial activity against *Staphylococcus* and *Streptococcus* bacteria strains.

Target compounds **VIII**-**XIII** were prepared via condensation of 2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione (**I**) with equimolar amounts of aromatic aldehydes **II**-**VII**. The product yields could be increased to 98% and more by carrying out the reaction in refluxing *i*-BuOH for 40 min.



I II – VII VIII – XIII

Ar = C_6H_5 (II, VIII); Ar = 4-$CH_3C_6H_4$ (III, IX); Ar = 4-$NO_2C_6H_4$ (IV, X); Ar = 3,4-$(CH_3O)_2C_6H_3$ (V, XI); Ar = 2-OHC_6H_4 (VI, XII); Ar = 4-BrC_6H_4(VII, XIII)

Green chemistry principles could be considered to have been satisfied by using environmentally benign *i*-BuOH and obtaining high yields from reactions (98 – 99%) [2].

The prepared 5-(arylmethylidene)-2,4,6-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones (**VIII**-**XIII**) were high melting colorless or colored compounds that were stable during storage, soluble in EtOH, DMSO, and $CHCl_3$ and slightly soluble in H_2O and C_6H_6.

The structures of the compounds were elucidated using IR, electronic, PMR, and ^{13}C NMR spectroscopy and mass spectrometry. Elemental analyses gave the compositions. IR spectra showed new absorption bands at 1625 – 1630 cm^{-1} for stretching vibrations of the ethylene bonds. PMR and ^{13}C NMR spectra exhibited singlets for the methine protons at 8.82 – 8.89 ppm and for methine C^7 at 135 – 140 ppm, respectively. Electronic spectra were characterized by two absorption bands with clearly defined maxima at 250 nm (local excitation of π-electrons) and 320 – 380 nm (intramolecular charge transfer characteristic of conjugated ethylenes [3]). Mass spectra of the synthesized compounds exhibited strong peaks for the molecular ions that enabled the molecular masses of the synthesized compounds to be estimated and peaks for fragments from primary and secondary dissociative ionization. Furthermore, mass spectra of the synthesized compounds contained a large number of additional peaks for ions that could be attributed to several empirical formulas so that a more detailed interpretation of the mass spectra was difficult.

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TABLE 1. Antimicrobial Activity of 5-(Arylmethylidene)-2,4,6(1*H*,3*H*,5*H*)-triones (**VIII-XIII**) Against *Staphylococcus* and *Streptococcus* Strains ($M \pm m$)

Compound	*Staphylococcus*, $n = 10$		Streptococcus, $n = 6$	
	MIC_{50}, μg/mL	MBC, μg/mL	MIC_{50}, μg/mL	MBC, μg/mL
VIII	3.2 ± 1.2	89.3 ± 14.1	$0.54 \pm 0.01^{***,###}$	$823.4 \pm 11.6^{###}$
IX	$523.6 \pm 4.1^{***,###}$	-	$548.6 \pm 3.6^{***,###}$	-
X	$45.3 \pm 3.8^{***,###}$	$998.5 \pm 2.6^{***,###}$	$28.6 \pm 1.4^{#}$	-
XI	$51.2 \pm 2.2^{***,###}$	$508.7 \pm 3.1^{***,###}$	$0.15 \pm 0.02^{***,###}$	$18.3 \pm 1.8^{##}$
XII	$51.2 \pm 1.1^{***,###}$	$501.6 \pm 2.4^{***,###}$	$0.27 \pm 0.03^{***,###}$	$986.7 \pm 10.1^{###}$
XIII	$6.4 \pm 1.9^{*,#}$	$200.8 \pm 8.1^{***,###}$	$0.49 \pm 0.01^{***,###}$	$894.3 \pm 8.4^{###}$
Cefazolin	1.2 ± 0.06	89.1 ± 3.2	16.3 ± 1.4	-
Ceftriaxone	0.7 ± 0.04	80.8 ± 1.4	8.2 ± 1.3	89.3 ± 2.1

* $p \le 0.05$, *** $p \le 0.01$ vs. cefazolin; $^{#}$ $p \le 0.05$, $^{##}$ $p \le 0.01$, $^{###}$ $p \le 0.001$ vs. ceftriaxone.

EXPERIMENTAL CHEMICAL PART

The target compounds were synthesized from 2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione (**I**) and aromatic aldehydes **II-VII** (chemically pure, Aldrich, USA). Their physical constants agreed with the literature [4]. IR spectra of the synthesized compounds were taken from KBr pellets on an InfraLUM FT-02 spectrometer in the range 4000 – 400 cm^{-1}. PMR and ^{13}C NMR spectra were recorded in DMSO-d_6 with HMDS internal standard on a Bruker DRX 500 SF instrument at operating frequencies 500 and 300 MHz, respectively. Electronic spectra of the compounds (0.3 mg/mL) in EtOH were recorded on a Cary-50 spectrophotometer. Mass spectra were obtained in a Finnigan SSQ 7000 mass spectrometer using direct sample introduction into the ion source, ionizing potential 70 eV at 500 – 550°C, and accelerating potential 5000 V (5000 resolution). The course of reactions and purity of products were monitored by ascending TLC on Silufol UV-254 plates using Me_2CO–hexane (2:3) and I_2 vapor [5]. Elemental analyses were performed on an automated Euro EA-3000 CHNS analyzer (Euro Vector, Germany).

5-(Arylmethylidene)-2,4,6-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones (VIII-XIII). A solution of **I** (10 mmol) and **II-VII** (10 mmol) in *i*-BuOH (25 mL) was refluxed for 40 min and cooled to room temperature. The precipitate was filtered off, rinsed with cold *i*-BuOH (2 × 15 mL), dried in air, and recrystallized from MeOH.

5-(Phenylmethylidene)-2,4,6-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (VIII). Yield 98%, mp 295 – 297°C [1].

5-[(4-Tolyl)methylidene]-2,4,6-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (IX). Yield 99%, mp (dec.) 275 – 278°C. IR spectrum, ν_{max}, cm^{-1}: 3550 (NH), 1770, 1750 (C=O), 1625 (C=C). PMR spectrum, δ, ppm: 11.25 (br, 1H, NH); 11.10 (br, 1H, NH); 8.35 – 7.15 (m, $4H_{arom}$, C_6H_4); 8.25 (s, 1H, CH); 2.35 (s, 3H, CH_3). ^{13}C NMR, δ, ppm: 163.9 (C_4), 163.4 (C_6), 155.2 (C_2), 150.2 (C_7), 140.5 (C_{11}), 133.1 (C_8), 130.4 (C_{10}), 128.3 (C_9), 120.3 (C_5), 21.6 (CH_3). UV spectrum, ν_{max}, nm: 260 (lgε 3.6), 380 (lgε 3.2). Mass spectrum, *m*/*z* (I_{rel}, %): 230 $[M]^+$ (100), 229 $[M-1]^+$ (58), 215 $[M-CH_3]^+$ (15), 187 $[M-CHNO]^+$ (28). $C_{12}H_{10}N_2O_3$.

5-[(4-Nitrophenyl)methylidene]-2,4,6-pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (X). Yield 98%, mp (dec.) 256 – 259°C. IR spectrum, ν_{max}, cm^{-1}: 3550 (NH), 1770, 1750 (C=O), 1625 (C=C), 1540 1365 (NO_2). PMR spectrum, δ, ppm: 11.22 (br, 1H, NH); 11.07 (br, 1H, NH); 8.25 – 7.51 (m, $4H_{arom}$, C_6H_4); 8.25 (s, 1H, CH). ^{13}C NMR, δ, ppm: 163.8 (C_4), 161.5 (C_6), 155.2 (C_2), 150.5 (C_7), 147.2 (C_{11}), 133.8 (C_8), 129.8 (C_{10}) 129.3 (C_9), 121.4 (C_5). UV spectrum, ν_{max}, nm: 260 (lgε 3.7), 380 (lgε 3.3). Mass spectrum, *m*/*z* (I_{rel}, %): 261 $[M]^+$ (100), 260 $[M-1]^+$ (55), 218 $[M-CHNO]^+$ (38). $C_{11}H_7N_3O_5$.

5-[(3, 4-Dimethoxyphenyl)methylidene]-2,4,6-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (XI). Yield 98%, mp (dec.) 298 – 302°C. IR spectrum, ν_{max}, cm^{-1}: 3550 (NH), 1770, 1750 (C=O), 1630 (C=C). PMR spectrum, δ, ppm: 11.45 (br, 1H, NH); 11.30 (br, 1H, NH); 7.40 – 7.15 (m, $3H_{arom}$, C_6H_3); 8.21 (s, 1H, CH); 3.80 (s, 3H, CH_3O); 3.79 (s, 3H, CH_3O). ^{13}C NMR, δ, ppm: 163.3 (C_4), 162.9 (C_6), 155.2 (C_2), 150.6 (C_7), 150.2 (C_{10}), 148.6 (C_{11}), 134.4 (C_8), 125.8 (C_{13}), 112.5 (C_{12}), 111.5 (C_9), 118.8 (C_5), 55.8 (CH_3O), 55.2 (CH_3O). UV spectrum, ν_{max}, nm: 260 (lgε 3.6), 375 (lgε 3.3). Mass spectrum, *m*/*z* (I_{rel}, %): 276 $[M]^+$ (100), 275 $[M-1]^+$ (52), 233 $[M-CHNO]^+$ (35). $C_{13}H_{12}N_2O_5$.

5-[(2-Hydroxyphenyl)methylidene]-2,4,6-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (XII). Yield 99%, mp (dec.) 290 – 293°C [1].

5-[(4-Bromophenyl)methylidene]-2,4,6-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (XIII). Yield 98%, mp (dec.) 248 – 252°C. IR spectrum, ν_{max}, cm^{-1}: 3550 (NH), 1770, 1750 (C=O), 1630 (C=C). PMR spectrum, δ, ppm: 11.50 (br,

1H, NH); 11.32 (br, 1H, NH); 8.15 – 7.90 (m, $4H_{arom}$, C_6H_4); 8.24 (s, 1H, CH). ^{13}C NMR, δ, ppm: 162.8 (C_4), 161.5 (C_6), 151.2 (C_2), 150.4 (C_7), 133.6 (C_8), 132.8 (C_{10}), 129.6 (C_9), 124.5 (C_{11}), 121.2 (C_5). UV spectrum, ν_{max}, nm: 260 (lgε 3.7), 360 (lgε 3.3). Mass spectrum, *m*/*z* (I_{rel}, %): 295 $[M]^+$ (100), 294 $[M-1]^+$ (53), 252 $[M-CHNO]^+$ (26). $C_{11}H_7BrN_2O_3$.

EXPERIMENTAL BIOLOGICAL PART

The series of 5-(arylmethylidene)-2,4,6-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones **VIII-XIII** were screened for microbiological activity against conditionally pathogenic strains *Staphylococcus aureus* 1899 (Research Institute of Genetics and Selection of Industrial Organisms, Moscow), *Streptococcus pyogenes* NIIL LSA (Leprosy Research Institute, Ministry of Health of the RF, Astrakhan), four strains of *Staphylococcus aureus*, five strains of *Staphylococcus epidermidis*, and five strains of *Streptococcus pyogenes* isolated from neurotrophic ulcers of leprosy patients (Leprosy Research Institute, Ministry of Health of the RF, Astrakhan) that were identified using a BIOMIC V3 digital imaging system (Giles Scientific, USA).

Serial dilutions were used for the screening [6]. Compounds dissolved in DMSO with concentrations decreasing by halves (from 128 to 0.25 μg/mL) were placed in order in tubes with liquid growth medium (meat-peptone broth). Each tube was treated with bacterial suspension (0.1 mL) of a certain density corresponding to standard turbidity 0.5 according to McFarland [7]. Inoculations were incubated in a thermostat at 37 ± 1°C for 24 h and then visually evaluated for the presence or absence of culture growth. The contents of the tubes were centrifuged at 1500 rpm for 10 min. The supernatant was discarded. The precipitate was sampled for inoculation on dense growth medium in a Petri dish containing egg-yolk high-salt agar for *S. aureus* and *S. epidermidis* or blood agar for *S. pyogenes* [8]. Grown colonies were counted on a BIOMIC V3 system after incubation for 1 d. The compound concentrations at which growth of colony-forming units (CFUs) was suppressed by 50% compared with a control (MIC_{50}) and by 100% (MBC) were determined.

The controls used inoculations of solvent (DMSO in equivalent volumes), inoculations without the compounds (positive control), and sterility tests (medium without inoculations and compounds). Compound activities were compared with those from inoculations of the strains with antibiotics of known activity, i.e., cefazolin and ceftriaxone (ZAO Rafarma, Russia) at concentrations identical to those of the compounds [9].

Results were processed statistically using the Student *t*-criterion. Table 1 presents the antimicrobial activities of **VIII** – **XIII** that were adjusted by subtracting the antimicrobial activity of DMSO (solvent).

Table 1 shows that the compounds had various activities against *Staphylococcus*. Compounds **VIII** and **XIII** had the highest antimicrobial activities with MIC_{50} values slightly greater than those of cefazolin and ceftriaxone. Their MIC_{50} values were consistently low despite the fact that these compounds had parameters that were inferior to those of the antibiotics. The MBC of **VIII** was statistically significantly the same as that of the reference drugs. Compounds **X**-**XII** under *in vitro* conditions at concentrations less than 45 – 52 μg/mL exhibited consistent bacteriostatic activity. However, *Staphylococcus* bacteria were less sensitive to **IX**.

The compounds also had various activities against *Streptococcus* bacteria (Table 1). Compounds **VIII**, **XI**, **XII**, and **XIII** showed pronounced bacteriostatic activity that was statistically significantly greater than that of the antibiotics. Compound **XI** was bactericidal at lower concentrations than ceftriaxone. The activity of **X** was comparable to that of cefazolin but statistically significantly less than that of ceftriaxone. Compound **IX** was less active also against *Staphylococcus*. Its MIC_{50} value was significantly greater than those of the antibiotics and the other tested compounds.

The mechanism of action of the compounds was not studied. However, the synthesized 5-(arylmethylidene)-2,4,6(1*H*,3*H*,5*H*)-triones could be considered compounds with chemical structures similar to the drug trimethoprim [10] because their molecules included a pyrimidine ring conjugated at the heterocyclic 5-position to an arylmethoxy group. This suggested that the mechanism of action of the synthesized compounds could be related to suppression of dihydrofolate reductase during dihydrofolic acid synthesis that led to depletion of the main cofactor for nucleic-acid synthesis, i.e., folate. As a result, protein and nucleic-acid production of the bacteria was disrupted [9]. In our opinion, the selective activity of the synthesized compounds was due to their ability to form significantly stronger chemical bonds to dihydrofolate reductase of the microorganisms than to this same enzyme of mammals [10].

Thus, the experimental results showed that 5-(arylmethylidene)-2,4,6-pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones **VIII-XIII** exhibited various bacteriostatic and bactericidal activities against *S. aureus*, *S. epidermidis*, and *S. pyogenes*. This made further studies of their biological activity critical.

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