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SYNTHESIS AND ANTIMYCOBACTERIAL ACTIVITY OF 5-(ARYLMETHYLENE)HEXAHYDROPYRIMIDINE-2,4,6-TRIONES

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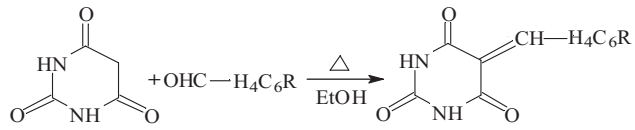
A series of 5-(arylmethylene)hexahydropyrimidine-2,4,6-triones were synthesized. Their antimycobacterial activity and acute daily toxicity with respect to *M. lufu* were investigated.

Keywords: synthesis, 5-(arylmethylene)hexahydropyrimidine-2,4,6-triones, antimycobacterial activity, minimum inhibiting and bactericidal concentrations, acute toxicity.

Several well-known antileprosy drugs are currently widely used in medical practice. However, their high toxicity [1, 2] and the development of resistance to them have shifted the first priority to the synthesis of novel antimycobacterial drugs. In our previous studies, the daily toxicity and antimycobacterial activity of substituted 2-nitro-1-(4-toluenesulfonyl)-2-[3-methyl(phenyl)-1,2,4-oxaliazol-5-yl]ethanes against *M. lufu* and *M. tuberculosis* were investigated [3].

In continuation of research and development in this area and in order to discover the least toxic compounds, we synthesized a series of 5-(arylmethylene)hexahydropyrimidine-2,4,6-triones (**VIII** – **XIII**) and investigated their antimycobacterial activity.



I II – VII VIII – XIII

R = H (II, VIII);
R = 4-CH_3O (III, IX);
R = 4-$(CH_3)_2N$ (IV, X);
R = 4-Cl (V, XI);
R = 3-NO_2 (VI, XII);
R = -OH(VII, XIII)

The method for preparing the target compounds (**VIII** – **XIII**), the yields of which were 82 – 92%, was based on condensation of 2,4,6-pyrimidinetrione (**I**) with an excess of the aromatic aldehydes (**II** – **VII**). The reaction was carried out in EtOH. The reaction mixture was refluxed for 45 min.

The structures of the compounds were established using IR and electronic spectroscopy, PMR and ^{13}C NMR spectra, and mass spectrometry. The compositions were determined by elemental analyses. IR spectra displayed a new absorption band that was missing in the starting materials for an ethylene bond at 1625 cm^{-1}. A methine singlet at 8.20 – 8.36 ppm in the PMR spectrum and a methine resonance for C_7 at 150 ppm in the ^{13}C NMR appeared characteristically. Electronic spectra showed two absorption bands with clearly resolved maxima at 260 nm (local π-electron excitation) and 350 – 450 nm (intramolecular charge transfer characteristic of conjugated ethylene [4]). Mass spectra of the synthesized compounds contained both very strong peaks for the molecular ions that allowed their molecular masses to be estimated and peaks for fragments from primary and secondary dissociative ionization. The hexahydropyrimidinetriones were high melting colorless or colored compounds that were soluble in most organic solvents and poorly soluble in H_2O.

EXPERIMENTAL CHEMICAL PART

The target compounds were synthesized from **I** and aromatic aldehydes (**II** – **VII**) (Aldrich, USA). Their physical constants agreed with the literature data. IR spectra of the synthesized compounds in KBr pellets were recorded in the range 4000 – 400 cm^{-1} on an InfraLUM FT-02 spectrophoto-

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meter. PMR and ^{13}C NMR spectra were recorded in DMSO-d_6 with HMDS internal standard on a Bruker DRX 500 SF at operating frequency 500 and 300 MHz, respectively. Electronic spectra (0.3 mg/mL in EtOH) were taken on a Cary-50 spectrophotometer. Mass spectra were obtained on a Finnigan SSQ 7000 mass spectrometer with direct sample introduction into the ion source at ionizing potential 70 eV, sample temperature 150°C, and accelerating potential 5000 V (resolution 5000). The course of reactions and purity of products were monitored by TLC on Silufol UV-254 plates using Me_2CO:hexane (2:3) and detection by I_2 vapor [5]. Elemental analyses were performed on a Euro Vector automated Euro EA-3000 CHNS analyzer and agreed with those calculated.

5-(Arylmethylene)hexahydropyrimidine-2,4,6-triones (VIII – XIII). A solution consisting of compound **I** (10 mmol) and **II** – **VII** (11 mmol) in EtOH (25 mL) was refluxed for 45 min and cooled to room temperature. The solid was filtered off, rinsed twice with cold EtOH (15 mL each), dried in air, and recrystallized from MeOH.

5-(Phenylmethylene)-2,4,6-pyrimidinetrione (VIII). Yield 85%, (dec.) 295 °C. IR spectrum, ν_{max}, cm^{-1}: 3550 (NH.), 1770, 1750 (C=O), 1625 (C=C). PMR spectrum, δ, ppm: 11.35 (br.s, 1H, NH); 11.20 (br.s, 1H, NH); 8.10 – 7.45 (m, $5H_{arom}$, C_6H_5); 8.30 (s, 1H, CH). ^{13}C NMR spectrum, δ, ppm: 163.4 (C_4), 161.6 (C_6), 154.7 (C_2), 150.2 (C_7), 133.1 (C_9), 132.7 (C_8), 132.2 (C_{10}), 128.0 (C_{11}), 119.1 (C_5). UV spectrum, λ_{max}, nm: 260 (lgε 3.5), 350 (lg ε 3.1). Mass spectrum, *m/z* (I_{rel}, %): 216 $[M]^+$ (70), 215 $[M-1]^+$ (100), 172 $[M\text{-}CHNO]^+$ (51), 102 $[C_8H_6]^+$ (15). $C_{11}H_8N_2O_3$. M 216.18.

5-[(4-Methoxyphenyl)methylene]-2,4,6-pyrimidinetrione (IX). Yield 87%, (dec.) 270 °C. IR spectrum, ν_{max}., cm^{-1}: 3550 (NH.), 1770, 1750 (C=O), 1625 (C=C). PMR spectrum, δ, ppm: 11.25 (br.s, 1H, NH); 11.10 (br.s, 1H, NH); 8.35 – 7.10 (m, $4H_{arom}$, C_6H_4); 8.25 (s, 1H, CH); 3.87 (s, 3H, CH_3O). ^{13}C NMR spectrum, δ, ppm: 163.9 (C_4), 163.4 (C_6), 162.2 (C_{11}), 155.0 (C_2), 150.2 (C_7), 137.5 (C_9), 125.2 (C_8), 115.5 (C_5), 113.9 (C_{10}), 55.7 (CH_3O). UV spectrum, λ_{max}., nm: 260 (lgε 3.6), 380 (lgε 3.2). Mass spectrum, *m/z* (I_{rel}, %): 246 $[M]^+$ (100), 245 $[M-1]^+$ (66), 215 $[M\text{-}CH_3O]^+$ (10), 202 $[M\text{-}CHNO]^+$ (38), 172 $[M\text{-}CHNO\text{-}CH_3O]^+$ (5),. $C_{12}H_{10}N_2O_4$. M 246.20.

5-[(4-Dimethylaminophenyl)methylene]-2,4,6-pyrimidinetrione (X). Yield 92%, (dec.) 277 °C. IR spectrum, ν_{max}, cm^{-1}: 3550 (NH.), 1770, 1750 (C=O), 1625 (C=C). PMR spectrum, δ, ppm: 11.20 (br.s, 1H, NH); 11.05 (br.s, 1H, NH); 9.60 – 8.30 (m, $4H_{arom}$, C_6H_4); 8.20 (s, 1H, CH); 2.95 (s, 6H, CH_3N). ^{13}C NMR spectrum, δ, ppm: 163.4 (C_4), 161.6 (C_6), 155.1 (C_2), 151.1 (C_{11}), 150.2 (C_7), 130.7 (C_9), 121.9 (C_8) 112.3 (C_{10}), 40.1 (CH_3N). UV spectrum, λ_{max}, nm: 260 (lgε 3.7), 450 (lgε 3.3). Mass spectrum, *m/z* (I_{rel}, %): 259 $[M]^+$ (100), 258 $[M-1]^+$ (53), 216 $[M\text{-}CHNO]^+$ (34), 215 $[M\text{-}(CH_3)_2N]^+$ (15). $C_{13}H_{13}N_3O_3$. M 259.26.

5-[(4-Chlorophenyl)methylene]-2,4,6-pyrimidinetrione (XI). Yield 90%, (dec.) 298 °C. IR spectrum, ν_{max}, cm^{-1}: 3550 (NH), 1770, 1750 (C=O), 1625 (C=C). PMR spectrum, δ, ppm: 11.45 (br.s, 1H, NH); 11.30 (br.s, 1H, NH); 8.15 – 7.55 (m, $4H_{arom}$, C_6H_4); 8.32 (s, 1H, CH). ^{13}C NMR spectrum, δ, ppm: 163.3 (C_4), 162.9 (C_6), 155.2 (C_2), 150.6 (C_7), 135.7 (C_{11}), 133.2 (C_8), 129.7 (C_{10}), 129.3 (C_9), 118.1 (C_5). UV spectrum, λ_{max}, nm: 260 (lgε 3.6), 350 (lgε 3.3). Mass spectrum, *m/z* (I_{rel}, %): 250 $[M]^+$ (100), 249 $[M-1]^+$ (42), 215 $[M\text{-}Cl]^+$ (10), 207 $[M\text{-}CHNO]^+$ (30). $C_{11}H_7N_2O_3Cl$. M 250.62.

5-[(3-Nitrophenyl)methylene]-2,4,6-pyrimidinetrione (XII). Yield 82%, (dec.) 245 °C. IR spectrum, ν_{max}, cm^{-1}: 3550 (NH), 1770, 1750 (C=O), 1625 (C=C), 1540, 1365 (NO_2). PMR spectrum, δ, ppm: 11.50 (br.s, 1H, NH); 11.35 (br.s, 1H, NH); 8.91 – 7.75 (m, $4H_{arom}$, C_6H_4); 8.36 (s, 1H, CH). ^{13}C NMR spectrum, δ, ppm: 162.8 (C_4), 161.5 (C_6), 151.2 (C_2), 150.2 (C_7), 147.2 (C_{12}), 138.4 (C_{13}), 134.5 (C_{10}), 129.4 (C_8), 126.1 (C_9), 125.5 (C_{11}), 121.6 (C_5). UV spectrum, λ_{max}, nm: 260 (lgε 3.7), 350 (lgε 3.3). Mass spectrum, *m/z* (I_{rel}, %): 261 $[M]^+$ (65), 260 $[M-1]^+$ (47), 244 $[M\text{-}NH]^+$ (100), 214 $[M\text{-}NO_2]^+$ (69), 172 $[M\text{-}NO_2\text{-}CHNO]^+$ (21). $C_{11}H_7N_3O_5$. M 261.16.

5-[(2-Hydroxyphenyl)methylene]-2,4,6-pyrimidinetrione (XIII). Yield 85%, (dec.) 290 °C. IR spectrum, ν_{max}, cm^{-1}: 3550 (NH), 3250 (OH), 1770, 1750 (C=O), 1625 (C=C). PMR spectrum, δ, ppm: 11.20 (br.s, 1H, NH); 11.05 (br.s, 1H, NH); 9.45 (br.s, 1H, OH), 8.05 – 7.05 (m, $4H_{arom}$, C_6H_4); 8.22 (s, 1H, CH). ^{13}C NMR spectrum, δ, ppm: 163.4 (C_4), 161.9 (C_6), 159.1 (C_9), 155.1 (C_2), 150.2 (C_7), 134.8

TABLE 1. Acute Daily Toxicity (LD_{50}) and Antimycobacterial Activity of 5-(Arylmethylene)hexahydropyrimidine-2,4,6-triones (**VIII** – **XIII**)

Compound	LD_{50}, mg/kg	Antimycobacterial activity	
		MIC, μg/mL	MBC, μg/mL
VIII	> 1500	2.5 ± 0.09***	8.1 ± 1.9*
IX	> 1500	42.8 ± 3.4**	64.0 ± 12.3**
X	1500	128.0 ± 11.6**	220.0 ± 19.8
XI	> 1500	2.3 ± 0.11**	7.65 ± 1.65*
XII	1500	256.0 ± 16.2***	428.5 ± 28.3**
XIII	1500	256.0 ± 18.9***	560.0 ± 26.4**
Dapsone	600	4.0 ± 0.7	8.3 ± 1.56

* Statistically significant differences relative to dapsone ($p \le 0.05$);
** statistically significant differences relative to dapsone ($p \le 0.01$);
*** statistically significant differences relative to dapsone ($p \le 0.001$).

(C_{11}), 132.9 (C_{13}), 121.2 (C_5), 120.1 (C_8), 118.3 (C12), 115.5 (C_{10}). UV spectrum, λ_{max}, nm: 260 (lgε 3.5), 350 (lgε 3.2). Mass spectrum, *m*/*z* (I_{rel}, %): 232 $[M]^+$ (100), 231 $[M - 1]^+$ (41), 215 $[M\text{-}OH]^+$ (18), 139 $[M\text{-}C_6H_5O]^+$ (54). $C_{11}H_8N_2O_4$. M 232.17.

EXPERIMENTAL BIOLOGICAL PART

Compounds **VIII** – **XIII** were screened microbiologically for antibacterial activity against *M. lufu* as a test culture for determining the *in vitro* activity of antileprosy drugs [6]. The procedure was based on the serial dilution method that was used to study antibacterial activity against *M. tuberculosis* [7]. *M. lufu* were cultivated in Lowenstein—Jensen dense medium for cultivation of mycobacteria [7]. The antimycobacterial activity of the compounds was studied in Shkol'nikova medium [7]. A two-week culture of *M. lufu* was seeded at a dose of 10^5 microbes per mL of medium. Cultures were synchronized in the cold (4 ± 1)°C for 72 h before seeding in order to obtain the most consistent results. The reference drug was dapsone (Novartis, Switzerland). Seeded samples were incubated with the compounds in Shkol'nikova medium for 10 d at (31 ± 1)°C. After this, suspension (0.05 mL) was transferred from each tube into tubes with slanted Lowenstein—Jensen medium in order to determine the viability of *M. lufu*. The remaining contents of the tubes were centrifuged. Smears were prepared from the precipitate and tested with Ziehl—Neelsen acid-fast stain [7]. Twenty fields-of-view were examined in the smears. The total number of mycobacteria was counted. Their morphology and the presence of acid-fast and mutated forms were assessed. Seedings in Lowenstein—Jensen medium were incubated for 10 d at (31 ± 1)°C. Colonies growing on dense medium were counted. The minimum inhibiting concentration (MIC) of the compounds was the lowest concentration at which the number of *M. lufu* colonies growing on the medium was less than 50% of the control value. The minimum bactericidal concentration (MBC) of the compounds was that concentration at which colonies of *M. lufu* were not observed after incubation in the medium. The investigation used five series of repeated experiments. The results were processed statistically using the Student *t*-criterion.

Acute toxicity (LD_{50}) was determined using male CBA mice (25 – 28 g) with seven animals per group. Compounds were injected into the stomach as an aqueous suspension at doses of 5 – 50 and 1500 mg/kg. LD_{50} values were calculated using the Miller and Tainter method and that of Gaddam [8]. All animals were synchronized with respect to housing and feeding and were observed for 10 d.

Table 1 presents the results for the antimycobacterial activity of **VIII** – **XIII**.

An analysis of the results in Table 1 showed that **VIII** – **XIII** possessed low toxicity (≥ 1500 mg/kg). The antimycobacterial activity of **VIII** – **XIII** against *M. lufu* varied widely. Compounds **VIII** and **XI** possessed the greatest antimycobacterial activity (MBA). Their activity was similar to that of dapsone. The differences between them were statistically insignificant ($p \le 0.05$). The activity of compound **XI** [MIC (2.3 ± 0.11) μg/mL] was statistically significantly ($p \le 0.01$) different from that of dapsone [MIC (4.0 ± 0.7) μg/mL] whereas that of **VIII** [MIC (2.5 ± 0.09) μg/mL] was statistically significantly greater ($p \le 0.001$). The other compounds were also active with respect to *M. lufu*. However, their MIC values were statistically significantly greater than that of dapsone ($p \le 0.01$; $p \le 0.001$).

Thus, the investigation showed that the compounds had low toxicity. The compounds exhibited antimycobacterial activity of varying degrees compared to that of dapsone. The results allowed **VIII** and **XI** to be considered promising for further studies of their antimycobacterial activity.

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