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ADENINE AND URACIL DERIVATIVES WITH ANTITUBERCULAR ACTIVITY

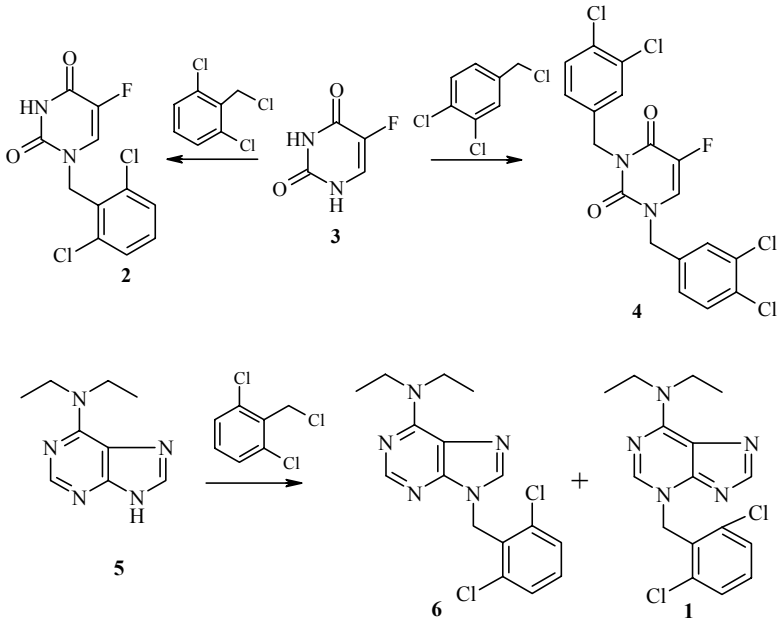
E. Alksnis, D. Korneeva, and E. Lukevics

We have carried out the synthesis and investigated the antitubercular activity of adenine and 5-fluorouracil derivatives. It was found that a comparatively large, lipophilic fragment is needed in the active molecule to inhibit the tuberculosis pathogen.

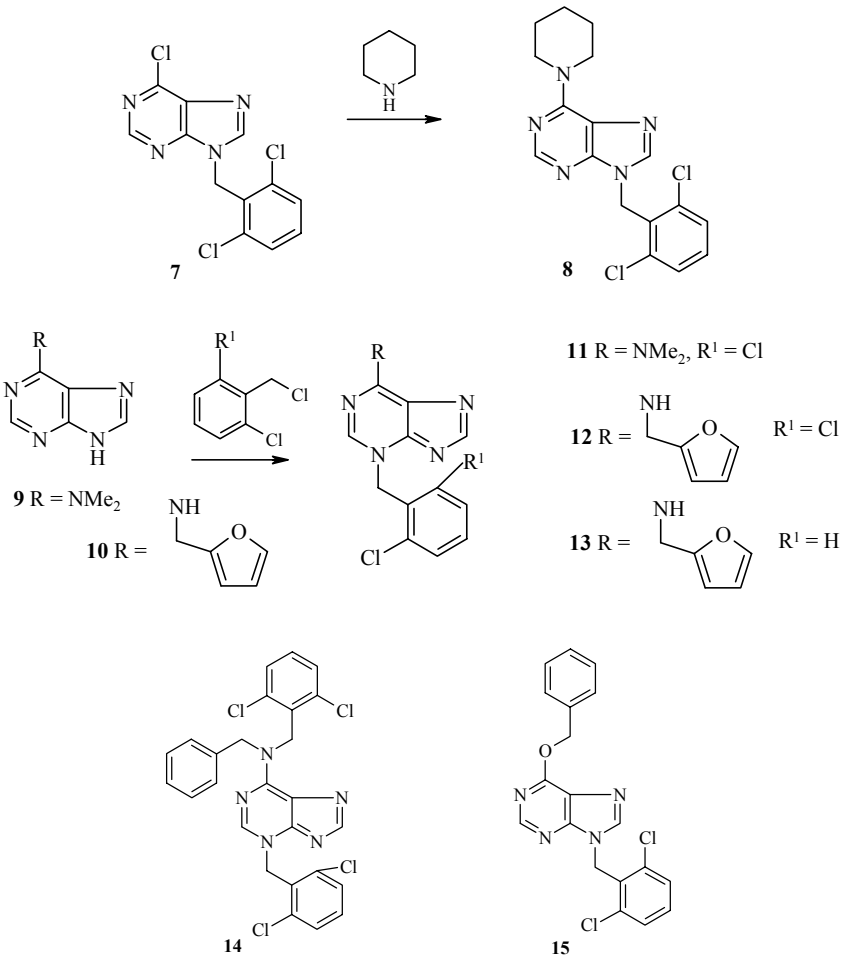
Keywords: pyrimidines, purines, alkylation, tuberculosis.

Tuberculosis is one of the most widespread and dangerous infectious diseases and the so called opportunistic tubercular infection is the main reason for the death of those who are ill with acquired immunodeficiency syndrome (AIDS). With the association that the tuberculosis stimulus rapidly acquires resistance to chemotherapeutic agents, it is constantly necessary to search for novel active preparations [1, 2].

The primary screening of the adenine, uracil, and xanthine derivatives synthesized by us was carried out in the USA under the Antimicrobial Acquisition and Coordinating Facility and showed compounds **1** and **2** to be characterized by limited antitubercular activity.



Latvian Institute of Organic Synthesis, Riga LV-1006; e-mail: pilsonis@osi.lv. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 6, pp. 807-810, June, 2001. Original article submitted October 11, 1998; revision submitted August 22, 2000.



We have further synthesized analogs of the compounds referred to (**4, 6, 8, 11-14**).

3(9)-Substituted derivatives of 6-diethylaminopurine **1, 6** and 5-fluorouracil **2, 4** were obtained by the alkylation of 6-diethylaminopurine (**5**) and 5-fluorouracil (**3**) using benzyl chlorides under phase-transfer catalytic conditions. The trisubstituted adenine derivative **14** was obtained in the same way. The mixtures of 3- and 9- substituted adenine derivatives **1, 6** were separated chromatographically on silica gel and the isomers were identified using UV and ^{1}H NMR spectroscopic methods as described in the study [3].

The 3-substituted adenine derivatives **11-13** were prepared by aralkylation of the adenines **9, 10** using benzyl chlorides in dimethylformamide and subsequent rearrangement in basic medium. The purine derivatives **8, 15** were synthesized from 6-chloro-9-(2,6-dichlorobenzyl)purine (**7**), similarly to the method reported in [3, 4].

Investigation of the antitubercular activity of the synthesized compounds (Table 1) has shown that the active substance molecule needs a comparatively large, lipophilic fragment differing in specific features for the efficient inhibition of the tuberculosis pathogen.

The most active of the compounds obtained (**8**) was 80 times inferior to the standard used in the experiment (rifampin). The structure of the novel compounds was proved using ^{1}H NMR spectroscopy (see Table 2).

TABLE 1. Antitubercular Activity of the Purine and Pyrimidine Derivatives

Compound	**8**	**12**	**2**	**6**	**13**	**1**	**4**	**11**	**14**	**15**
Inhibition *Mycobacterium tuberculosis* H37Rv, %*	73	53	44	31	20	19	13	4	0	0

* At a concentration of 12.5 μg/ml.

TABLE 2. ^{1}H NMR Spectra of the Purine and Pyrimidine Derivatives

Compound	Chemical shift, δ, ppm ($CDCl_3$)
1	1.20 (6H, t, CH_3); 3.86 (4H, q, CH_2); 5.49 (2H, s, CH_2); 7.11-7.42 (4H, m, Ph, purine ring); 8.27 (1H, s, purine ring)
2*	5.03 (2H, s, CH_2); 7.43 (3H, m, Ph); 7.62 (1H, d, pyrimidine ring); 11.72 (1H, br. s, NH)
4	4.76 (2H, s, CH_2); 4.99 (2H, s, CH_2); 6.93-7.54 (7H, m, Ph, pyrimidine ring)
6	1.50 (6H, m, $2CH_3$); 3.73 (2H, m, $N_{(6)}CH_2$); 4.40 (2H, m, $N_{(6)}CH_2$); 5.82 (2H, s, CH_2); 7.16-7.53 (4H, m, Ph, purine ring); 8.00 (1H, s, purine ring)
8	1.65 (6H, s, CH_2); 4.17 (4H, s, $N_{(6)}CH_2$); 5.54 (2H, s, CH_2); 7.15-7.44 (4H, m, Ph, purine ring); 8.32 (1H, s, purine ring)
12	4.79 (2H, br. s, $N_{(6)}CH_2$); 5.83 (2H, s, CH_2); 6.19 (2H, m, furan ring); 7.05-7.45 (4H, m, Ph, furan ring); 7.60 (s); 7.96 (s) (2H, purine ring)
13	4.80 (2H, br. s, $N_{(6)}CH_2$); 5.62 (2H, s, CH_2); 6.20 (2H, m, furan ring); 7.04-7.46 (5H, m, Ph, furan ring); 7.60 (s), 7.96 (s) (2H, purine ring)

* In DMSO-d_6.

EXPERIMENTAL

^{1}H NMR spectra were taken on a Bruker WH-90 spectrometer using TMS as internal standard. Melting points were determined on a Boetius apparatus and are not corrected. The ultraviolet spectra were recorded on a UNICAM UV-vis spectrophotometer. TLC analysis was carried out on Silufol UV-254 plates in the systems A: chloroform–ethyl acetate, 1:1 and B: chloroform–ethyl acetate–ethanol, 2: 2: 1. The column chromatography was performed on L40/100 grade silica gel in the same systems.

The synthesis of the purines **7** and **15** has been reported in the study [3] and of compound **14** in [4].

The antitubercular activity for the compounds prepared was studied in the culture *Mycobacterium tuberculosis* H37Rv in the medium BACTEC 12B with the radiometric system BACTEC 460.

9-(2,6-Dichlorobenzyl)-6-diethylaminopurine (1) and 3-(2,6-Dichlorobenzyl)-6-diethylaminopurine (6). A mixture of 6-diethylaminopurine **5** (1.91 g, 10 mmol), benzene (20 ml), aqueous NaOH solution (50%, 8 ml), tetrabutylammonium bromide (0.32 g, 1 mmol), and 2,6-dichlorobenzyl chloride (2.14 g, 11 mmol) was heated at 80°C until solution of the suspension of the purine sodium salt (30-45 min). The mixture was cooled, water (100 ml) added, and twice extracted with chloroform (50 ml). The chloroform extracts were dried over anhydrous sodium sulfate and evaporated to dryness and the dry residue was separated on a silica column (30 × 2 cm) using system A. Following chromatography, crystallization from hexane gave compound **1** (2.48 g, 71%) and compound **6** (0.63 g, 18%); mp 201-202°C, λ_{max} 309 nm (methanol). **Compound 1.** Found, %: N 20.08; C 54.93; H 4.88. $C_{16}H_{17}Cl_2N_5$. Calculated, %: N 20.00; C 54.86; H 4.86. **Compound 6.** Found, %: N 20.17; C 55.11; H 4.86. $C_{16}H_{17}Cl_2N_5$. Calculated, %: N 20.00; C 54.86; H 4.86.

6-Piperidino-9-(2,6-dichlorobenzyl)purine (8). 6-Chloro-9-(2,6-dichlorobenzyl)purine **7** (0.32 g, 1 mmol) and piperidine (2.55 g, 30 mmol) were heated at 100°C for 2 h. The solution was poured into water (50 ml) and after 2 h it was filtered, washed with water, and crystallized from ethanol to give compound **8** (0.15 g, 41%); mp 191-192°C, λ_{max} = 280 nm (methanol). Found, %: N 19.42; C 56.45; H 4.77. $C_{17}H_{17}Cl_2N_5$. Calculated, %: N 19.32; C 56.37; H 4.74.

3-(2,6-Dichlorobenzyl)-6-dimethylaminopurine (11), 3-(2,6-dichlorobenzyl)-6-furfurylaminopurine (12), and 3-(2-chlorobenzyl)-6-furfurylaminopurine (13). (General Method). A suspension containing the aminopurine (10 mmol), benzyl chloride (10 mmol), and dimethylformamide (10 ml) was heated at 110°C for 2 h. Water (10 ml) was added and the product was neutralized with concentrated ammonia solution. NaOH solution (1N, 50 ml) was added and the product was twice extracted with chloroform. The chloroform extracts were dried over anhydrous sodium sulfate and evaporated. The dried residue was chromatographed on a silica gel column (30 × 4 cm, system A, then B). After chromatography the products were crystallized from ether to give compound **11** (1.96 g, 61%); mp 212-213°C, λ_{max} 309 nm (methanol), compound **12** (1.83 g, 49%); mp 171-173°C, λ_{max} 294 nm (methanol), and compound **13** (1.80 g, 53%); mp 191-193°C, λ_{max} 294 nm (methanol). **Compound 11**. Found, %: N 21.58; C 54.20; H 4.11. $C_{14}H_{13}Cl_2N_5$. Calculated, %: N 21.74; C 52.19; H 4.07. **Compound 12**. Found, %: N 18.55; C 54.62; H 3.50. $C_{17}H_{13}Cl_2N_5O$. Calculated, %: N 18.71; C 54.56; H 3.51. **Compound 13.** Found, %: N 20.45; C 60.28; H 4.19. $C_{17}H_{14}ClN_5O$. Calculated, %: N 20.61; C 60.28; H 4.15.

5-Fluoro-1-(2,6-dichlorobenzyl)uracil (2). A suspension containing 5-fluorouracil (1.3 g, 10 mmol), powdered KOH (0.56 g, 10 mmol), benzene (10 ml), and trioctylmethylammonium bromide (0.4 g) was stirred for 1 h at 80°C. 2,6-Dichlorobenzyl chloride (1.95 g, 10 mmol) was added and the product was heated and stirred for a further 2 h. The mixture was cooled and washed with benzene. The residue was twice extracted with hot chloroform (50 ml). The chloroform extracts were evaporated and the residue was crystallized from butanol to give compound **2** (0.6 g, 21%); mp 253-255°C, λ_{max} 273 nm (methanol). Found, %: N 9.69; C 45.70; H 2.44. $C_{11}H_7Cl_2FN_2O_2$. Calculated, %: N 9.61; C 45.63; H 2.46.

1,3-Di(3,4-dichlorobenzyl)-5-fluorouracil (4). A two phase system containing 5-fluorouracil (1.3 g, 10 mmol), sodium hydroxide (0.8 g, 20 mmol), water (8 ml), tetrabutylammonium bromide (0.64 g, 2 mmol), and 3,4-dichlorobenzyl chloride (3.9 g, 20 mmol), heated at 60°C, was stirred for 1.5 h. Sodium hydroxide solution (1 N, 50 ml) was added and the product was twice extracted with chloroform (50 ml). The chloroform extracts were dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. Recrystallization from acetonitrile gave compound **4** (2.3 g, 52%); mp 142-144°C, λ_{max} 273 nm (methanol). Found, %: N 6.36; C 48.00; H 2.52. $C_{18}H_{11}Cl_4FN_2O_2$. Calculated, %: N 6.25; C 48.25; H 2.47.

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