SYNTHESIS AND BIOLOGICAL ACTIVITY OF 1-(5-FLUOROURACIL-1-YL)-1-(α-ADAMANTYL-1-ETHYLIMINO)ETHANE

É. Sh. Mamedov, R. Ya. Akhverdieva,
Z. A. Lazymova, T. A. Asadullaev,
and R. A. Babakhanov

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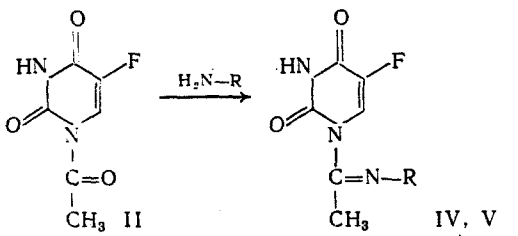
In continuation of our studies on the synthesis of potentially antiviral and antibacterial compounds from the adamantane group, and the establishment of a relationship between their structure and biological activity, we synthesized certain derivatives of such as 5-fluorouracil (I), 1-acetyl-5-fluorouracil (II), 1,3-diacetyl-5-fluorouracil (III), 1-acetohydrazonoyl-5-fluorouracil (IV), and 1-(5-fluorouracil-1-yl)-1-(α-adamantyl-1-ethylimino)-ethane. The reason for the synthesis of the last compound was the presence of pronounced antiviral properties in 5-fluorouracil and its derivatives [1].

Compounds II and III were obtained by heating I in excess of Ac_2O, compounds IV and V by the reaction of II with hydrazine hydrate and α-methyl-1-adamantylmethylamine hydrochloride (remantadine, VI) under mild conditions without splitting of the uracil ring observed in similar reactions when the mixture was heated above 60°C [4].



R = NH_2 (IV); 1-(1-adamantyl)ethyl (V)

Endo-effects with maxima at 160 and 265°C, are observed on the thermogram of compound IV. At a temperature of up to 200°C, ions with m/z 28, corresponding to molecular nitrogen, are found in the mass spectrum. In the IR spectra of compounds IV and V, the intense absorption bands at 1700 and 1710 cm^{-1} correspond to the stretching vibrations of the C=O bond in the uracil fragment. The absorption band at 1635-1645 cm^{-1} belongs to the stretching vibrations of the C=N bond of the hydrazone form [3]. Other bands, confirming the structure of the compounds synthesized were also found in the IR and PMR spectra.

In a study of the antiviral activity of compounds II and V, it was found that II has a statistically unreliable activity. In contrast to II, compound V exhibited a high antiviral activity, exceeding the similar activity of I and VI. Compound V has also bacteriostatic activity.

EXPERIMENTAL (CHEMICAL)

The mass spectra were run on a MX-1303 spectrometer with direct introduction of the sample into the ionization region at an energy of ionizing electrons of 50 eV. The thermal stability was studied on a brand MOM derivatograph in the temperature range of 15-570°C in an air atmosphere, and at a heating rate of 10 degrees/min. The IR spectra were run on a UR-20 spectrophotometer in the form of a suspension in mineral oil, and the PMR spectra, on a Tesla BS 487B spectrometer. The chromatography was carried out on Silufol UV-254 plates in a AcOH—benzene—ethanol, 10:9:6, system and the spots were developed by UV irradiation.

Institute of Theoretical Problems of Chemical Engineering, Academy of Sciences of the Azerbaidzhan SSR. G. M. Musabekov Scientific Research Institute of Virology, Microbiology and Hygiene of the Ministry of Public Health of the Azerbaidzhan SSR. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 22, No. 1, pp. 57-59, January, 1988. Original article submitted June 24, 1986.

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TABLE 1. Antiviral Activity of Compounds I, II, V, and VI

Compound	Degree of infection by virus in cells culture, EID_{50}	Degree of infection by virus in chicken embryos, EID_{50}
Control virus	8,5±0,25	9,0±0,30
I	6,0±0,21	7,5±0,15
II	7,7±0,3	8,0±0,22
V	4,0±0,15	4,5±0,20
VI	6,0±0,20	6,0±0,18

1-Acetyl-5-fluorouracil (II) and 1,3-Diacetyl-5-fluorouracil (III). A mixture of 5.2 g (40 mmoles) of I and 50 ml of Ac_2O is heated for 4 h at 140°C. Acetic anhydride is distilled off at reduced pressure, and the white precipitate obtained (6.94 g) is washed with ether. when the ether is evaporated, 0.7 g (8.2%) of III is obtained, mp 81-82°C, R_f 0.64. IR spectrum, ν_{max}, cm^{-1}: 1710 (C=O). PMR spectrum (D_2O), δ, ppm: 2.38 s, 2.84 s (CH_3), 7.8-8.0 d, J 6.6 Hz (CH=CF). Found, %: C 44.97; H 3.19; F 8.95; N 13.21. $C_8H_7FN_2O_4$. Calculated, %: C 44.86; H 3.27; F 8.88; N 13.08. The remaining precipitate (6.24 g) is dissolved in acetone. The solution obtained is filtered, and the filtrate is evaporated to yield 6 g (87.2%) of II, mp 124-125°C, R_f 0.71. IR spectrum, ν_{max}, cm^{-1}: 1715 (C=O). PMR spectrum (d_6 = DMSO), δ, ppm: 2.62 s (CH_3), 8.2-8.3 d J 6.8 Hz (CH=CF). Found, %: C 41.75; H 2.89; F 11.12; N 16.36. $C_6H_5FN_2O_3$. Calculated, %: C 41.86; H 2.91; F 11.05; N 16.27.

1-Acetohydrazonoyl-5-fluorouracil (IV). A 0.5 g portion (8.3 mmoles) of AcOH is added to 3.45 g (69 mmoles) of hydrazine hydrate. A solution of 3 g (23 mmoles) of II in 30 ml of alcohol is added dropwise in the course of 30 min, with stirring, to this solution. At the end of the addition, the mixture is stirred at room temperature for 30 min. The precipitate is filtered, and the filtrate evaporated. The precipitate (3.75 g) obtained by filtration of the reaction mixture and evaporation of the filtrate, is washed with MeOH and recrystallized from ethanol to yield 2.92 g (90%) of IV, mp 160°C (dec). R_f 0.87. IR spectrum, ν_{max}, cm^{-1}: 1635-1645 (C=N), 1700 (C=O), 3140 (NH), 3320 (NH_2), PMR spectrum (d_6 = DMSO), δ, ppm: 2.34 s (CH_3), 5.12 br. s (NNH_2), 8.1-8.2 d, J 6.8 Hz (CH=CF). Found, %: C 38.78; H 3.81; F 10.29; N 30.38. $C_6H_7FN_4O_2$. Calculated, %: C 38.78; H 3.76; F 10.22; N 30.11.

1-(5-Fluorouracil-1-yl)-1-(adamantyl-1-ethylimino)ethane (V). A solution of 0.79 g (4.6 mmoles) of compound II in 15 ml of ethanol is added slowly dropwise, with stirring, to a solution of 1 g (4.6 mmoles) of VI in 14 ml of ethanol and 0.47 g (4.65 mmole) [sic]. At the end of the addition, the reaction mixture is heated at 40°C for 1 h. The solvent is evaporated, and the precipitate is washed with $CHCl_3$. The residue (1.04 g, 67%) is compound V, mp 220-221°C (dec). R_f 0.67. IR spectrum, ν_{max}, cm^{-1}: 1635-1640 (C=N), 1710 (C=O), 3160 (NH). PMR spectrum (d_6 = DMSO), δ, ppm: 1.39-2.64 m (adamantyl), 1.24 d, 3.46 m, J 7 Hz (CH_3CH), 2.02 (CH_3C), 7.8-8.0 d, J 6.6 Hz (CH=CF). Found, %: 64.78; H 7.48; F 5.78; N 12.54. $C_{18}H_{24}FN_3O_2$. Calculated, %: C 64.86; H 7.21; F 5.71; N 12.61.

EXPERIMENTAL (BIOLOGICAL)

The antiviral activity was studied in a culture of both primary trypsinylated cells of chicken fibroblasts and of chicken embryos on a model of a Waybridge strain of an influenza virus of a fowl pestilence (antigenic formula H7N7) with an infection titer of 8.5 lg $EID_{50}/0_2$ ml and with a hemaglutinating activity of 640-1280 HAU/ml. In both cases, the compounds are introduced 1 h before the infection and at the moment of the infection. The concentration of the compounds under in vitro conditions was 5 μg/ml, and in ovo it was 1 mg/embryo for VI, and 50 μg/ml for I and V. The standard deviation and the reliability of the divergence between the control and experimental data was determined by the method in [2].

First, the action of the compounds on the reproduction of the influenza virus in the cell culture was studied. The cells were cultivated at 37°C on a medium 199, enriched by

a 10% bovine serum. After the infection, a monolayer of the cells was washed out by a Hanks solution and flooded with suspension medium with the addition of antibiotics. The multiplicity of infection was 100 TCD_{50}. The compounds were introduced into the culture medium 1 h before the 30-min adsorption of the virus at 37°C and immediately after the infection. After 24 h, the culture liquor was decanted, and the infection activity of the virus was determined by the method in [5].

The results are listed in Table 1.

Table 1 shows that preliminary treatment of the infected monolayer with a compound, followed by its introduction in a concentration of 5 μg/ml, leads to the inhibition of the reproduction of the virus. In the case of I, the infection titer of the virus decreases by 1.5 log, of II by 0.8 log, of VI by 2.5 log, and of compound V by 4.5 log.

The activity of compounds I, II, V, VI was then studied in developing chicken embryos. Table 1 shows that the inhibiting effect of I and VI was 1.5 and 3.0 log at an infection multiplicity of 1000 EID. Under these conditions, compound V led to a stronger suppression of the reproduction of the fowl pestilence virus, compared with I and VI (a 4.5 log decrease).

The antibacterial activity of the compounds was studied by the method of diffusion in agar. The bacterial culture in an inoculation dose of 250 million cells in 1 ml was deposited on the surface of a solidified meat-peptone agar. The agar was dried, and discs of filter paper (10 mm in diameter) impregnated with the compounds were placed on the infected surface of the agar. The antibacterial activity was indicated by the diameter of the growth inhibition zone. Compound IV does not inhibit the development of the bacterial cultures. The diameter of the growth inhibition zone (mm) for compounds I and V is 23 and 15, respectively, on the *Staph. aureus* culture, 17 and 15 on *E. coli*, 0 and 15 on Newport, 27 and 15 on *Sal. typhi*.

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