Pharmaceutical Chemistry Journal, Vol. 28, No. 4, 1994

SEARCH FOR NEW DRUGS

SYNTHESIS OF N^1-SUBSTITUTED 6-AZACYTOSINES AND THEIR BIOLOGICAL ACTIVITY

I. V. Alekseeva, L. I. Pal'chikovskaya, A. S. Shalamai, S. S. Ognyanik, N. V. Morgart, and N. A. Petrusha

UDC 615.31+547.963.3

The synthesis of biologically active compounds — derivatives and analogs of 6-azacytosine riboside (6-azacytidine), has drawn the attention of research workers in connection with the search for antileucotic preparations of low toxicity having immunotropic properties [1].

In the present work the development is reported of an effective method of preparation of N^1-tetrahydrofuranyl derivatives of 6-azacytosines and other azapyrimidine bases, their glycosides and also comparative data are given on the antileucotic action of three derivatives of 6-azacytosine derivatives on animals.

The known methods of introduction of the tetrahydrofuranyl residue into pyrimidine heterocycles by alkylation of the corresponding trimethylsilyl ethers do not give positive results for the azapyrimidine series [2, 3]. We succeeded in shortening the reaction time and improving the isolation conditions in comparison with the previously described methods [3] using a simplified variant of the synthesis proposed for the preparation of 6-azapyrimidine nucleosides [4]. We showed that by using the process of alkylation of substituted azapyrimidines by 1-acetoxytetrahydrofuran combined with silylation it is possible to obtain N^1-substituted derivatives (I-V) in good yields (see Table 1). The furanylation proceeds most effectively in the case of 4-thio-azapyrimidines I, II, which can be readily converted into the corresponding derivatives of 6-azacytosine III-IV. N-(2-Tetrahydrofuranyl)-N^4-carbamoylmethyl-6-azacytosine V was obtained in a similar way by the reaction of glycinamide with I in DMFA.

Under other reaction conditions, for example, those providing for the preparation of 1-acetoxytetrahydrofuran from dihydrofuran and acetic anhydride *in situ*, we were unable to obtain the expected N^1-tetrahydrofuranyl-substituted products.

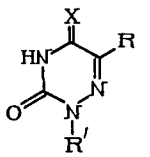
The directivity of the alkylation reaction of azapyrimidines was studied by means of UV and PMR spectroscopy methods. It had previously been found that the criterion determining the position of a substituent at the N^1-atom of the azapyrimidine ring is the absence of a bathochromic shift of the absorption maximum in an alkaline medium [5]. On comparison of the spectral characteristics of the tetrahydrofuranyl derivatives of 6-azacytosine III-IV and the previously synthesized nucleosides IIIa, IVa, a common pattern was noted in their UV absorption depending on the pH of the medium (see Table 2). Thus, in the spectra of the above compounds taken at pH 10-12, the long-wave shift of λ_{max} was not observed, so that it was possible to ascribe the structure of N^1-derivatives to the tetrahydrofuranyl-substituted derivatives III, IV.

In the study of the PMR spectra of the synthesized compounds it was found that the signals of the NH protons of the azacytosine derivatives III, IV are recorded in the form of two singlets in the 7.0-8.0 ppm region. This disposition of signals may occur in the case of nonequivalent protons of an exo-amino group or, for example, during the realization of an imine structure in which the descreening effects of the neighboring atoms are lessened. The PMR spectrum of the N^3-alkyl substituted 6-azacytosine obtained by a directed synthesis can also serve as an evidence for the imine structure of derivatives III, IV. The proton signal at the N^4-exocyclic atom in this compound is observed at 8.62 ppm, while the signal of the proton present at the N^1 atom of the heterocyclic ring is observed in the weak field (11.70 ppm), as happens in the case of other N^3-substituted natural derivatives of azapyrimidine [5].

Institute of Molecular Biology and Genetics, Academy of Sciences of the Ukraine, Institute of Experimental Pathology, Oncology, and Radiobiology, Academy of Sciences of Ukraine, Kiev. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 28, No. 4, pp. 16-18, April, 1994. Original article submitted July 2, 1993.

0091-150X/94/2804-0227$12.50

TABLE 1. Synthesized Compounds



I–V

Compound	X	R	R'*
I	S	H	THF
II	S	Me	THF
III	NH	H	THF
IIIa	NH	H	Rib
IV	NH	Me	THF
IVa	NH	Me	Rib
V	NCH_2CONH_2	H	THF

*THF) The tetrahydrofuranyl radical; Rib) the ribofuranose residue.

The problem of the amino–imino tautomerism of the 6-azacytosine has been discussed previously [6], but still has not found a complete solution, and requires further investigations.

EXPERIMENTAL (CHEMICAL)

The characteristics of the synthesized compounds are given in Table 2. The UV spectra were run on a "Specord UV-vis" spectrophotometer, the PMR spectra — in DMSO-d_6 on a "Varian-Gemini-200" spectrometer, using TMS as an internal standard. The purity of the obtained compounds was monitored by TLC on Kieselgel-60-F_{254} plates from "Merck AG" in the systems of solvents $CHCl_3$–MeOH (9:1), A; butanol–acetic acid–water (4:1:5), B. The elemental analysis data of compounds I-V correspond to the calculated values.

N^1-(2-Tetrahydrofuranyl)-4-thio-6-azauracil (I). A 14 ml portion (62 mmoles) of hexamethyldisilazane, 8 ml (62 mmoles) of trimethylchlorosilane, and 7 ml (60 moles) of $SnCl_4$ were added to 10.4 g (80 mmoles) of 4-thio-6-azauracil [4] in 400 ml of a dry acetonitrile, and the suspension was stirred to the complete dissolution of the starting materials (15-20 min). The mixture was cooled to 0°C and a solution of acetoxytetrahydrofuran (17.2 ml, 120 mmoles) in acetonitrile was added to the mixture in an argon current. The reaction mixture was stirred without cooling for 2 h. At the end of the reaction 5 ml of ethanol was added, after 30 min the precipitate of the unreacted heterocyclic compound was filtered off, and the solvent was evaporated. The residue was deposited on a column with silica gel, and eluted with the $CHCl_3$–MeOH mixture (gradient).

N^1-(2-Tetrahydrofuranyl)-5-methyl-4-thio-6-azathymine (II) was obtained from 1.4 g of 4-thio-6-azathymine [4] and 1.75 ml of 1-acetoxytetrahydrofuran under the conditions of the synthesis of I. The yield of II was 1.85 g, R_f 0.8 (A).

N^1-(2-Tetrahydrofuranyl)-6-azacytosine (III). Method A. A solution of 0.33 g (1.5 mmole of compound I in 15 ml of propanol was boiled for 3 h in a current of gaseous ammonia. The solvent was distilled off, the residue was washed with chloroform and dried. Product III was purified by crystallization from water. Yield, 0.28 g, R_f 0.3 (A); 0.53 (B). Compound IV was obtained in a similar way from compound II, R_f 0.35 (A); 0.6 (B).

Method B. The synthesis was carried out in a similar way by the above-described procedure for I. The raw product was purified by crystallization from an aqueous ethanol.

N^1-(2-Tetrahydrofuranyl)-N^4-carbamoylmethyl-6-azacytosine (V). A 0.52 g portion (2.5 mmoles) of compound I in 6 ml of DMFA was heated for 4 h at 90°C with 0.37 g (5 mmoles) of glycinamide. The solvent was distilled off. The residue was purified on a column with silica gel, eluent — using a concentration gradient of ethanol in chloroform (0-20%) as eluent. Yield, 0.3 g of compound V, R_f 0.2 (A); 0.43 (B).

TABLE 2. Physicochemical Characteristics of the Synthesized Compounds

Compound	Yield, %	Mp,	Empirical formula	UV spectrum, λ_{max} (log ε)			PMR spectrum, δ, ppm	
				3 N HCl	H_2O	0.01 N KOH	N^1H	
I	83	143—4	$C_7H_9N_3O_2$	243(3,86) 332(4,20)	245(3,85) 332(4,16)	247(3,93) 334(4,14)	13,63	
II	87	136—7	$C_8H_{11}N_3O_2$	239(3,60) 331(4,06)	238(3,52) 331(4,02)	240(3,82) 332(4,03)	13,708	
III	72	208—10	$C_7H_{10}N_4O_2$	284(3,86)	267(3,85)	266(3,87)	7,94;	7,84
IIIa	76	216—8	$C_8H_{12}N_4O_5$	285(3,86)	264(3,86)	264(3,87)	8,02;	7,91
IV	76	196—8	$C_8H_{12}N_4O_2$	289(3,83)	262(3,85)	259(3,85)	7,98;	7,42
IVa	65	238—9	$C_9H_{14}N_4O_5$	289(3,81)	260(3,90)	260(3,97)	8,01;	7,45
V	52	111—2	$C_9H_{13}N_5O_3$	284(3,81)	273(3,84)	275(3,83)	7,55;	7,18

N^1-(β-D-Ribofuranosyl)-6-azacytosine (IIIa). An 18 ml portion of hexamethyldisilazane, 35 ml of trimethylchlorosilane, and 18 ml (0.135 mole) of $SnCl_4$ were added to a mixture of 12.9 g (0.1 mole) of 4-thio-6-azauracil and 41.3 g (0.13 mole) of tetraacetylribofuranose in 1 liter of acetonitrile. The reaction mixture was allowed to stand for 3 h at room temperature, was then diluted with 300 ml of $CHCl_3$ and 100 ml of water. The organic layer was separated, washed with an $NaHCO_3$ solution, water, dried over Na_2SO_4, and evaporated under vacuum to dryness. The residue was dissolved in chloroform (50 ml) and the solution was filtered through a layer of inactivated silica gel. The filtrate was evaporated. The oily residue was dissolved in 300 ml of a dry ethanol, and an ammonia current was passed through the boiling solution for 3.5 h. The mixture was cooled, diluted with 100 ml of a 25% solution of NH_4OH, and allowed to stand for 24 h. After evaporation of the reaction mixture, the residue was crystallized from an aqueous ethanol. The yield of compound IIIa was 18.5 g, R_f 0.28 (B).

N^1-(β-D-Ribofuranosyl)-5-methyl-6-azacytosine (IVa) [7] was obtained similarly to the above described synthesis of IIIa from 7 g (0.05 mole) of 4-thio-6-azathymine, 24 g (0.07 mole) of tetraacetylribofuranose in the presence of 11 ml (0.05 mole) of hexamethyldisilazane and 10 ml (0.07 mole) of $SnCl_4$. The yield of IVa was 10.5 g, R_f 0.32 (B).

EXPERIMENTAL (BIOLOGICAL)

The antileucotic effect of the synthesized 6-azacytosine derivatives was determined on hybrid mice BDF_1 and on mice of the DBA_2 line (each weighing 20-25 g) with a model leucosis L-1210 by a method accepted in the chemotherapy of leucoses. The implantation of the leucosis was carried out in the abdominal cavity (10^5-10^6 cells per mouse). The treatment was started 24 h after the implantation and was continued for 5-6 days. The criterion for the activity was the index of the mean increase in the survival of the animals (ISA). The toxicity parameters by the rapid method of Frumin [8] and the course doses were preliminarily determined for the individual preparations.

The LD_{50} for compound III was 2400-2500 mg/kg for compound III, 9250-9400 mg/kg for compound IIIa, and 7165 mg/kg for compound IVa.

In the study of the antileucotic action, compound III was used in a dose from 50 to 500 mg/kg, compound IIIa — from 150 to 1500 mg/kg, and compound IVa — from 600 to 1200 mg/kg.

It was found that 6-azacytidine in doses of 150, 600, 800, and 900 mg/kg at intraperitoneal administration inhibits the development of leucosis L-1210 by 36, 41, 57, and 69%, respectively. During the intravenous administration of this preparation, the ISA of mice with leucosis increased to 82%. On administration of compound III, the highest therapeutic effect was observed at doses of 230 and 300 mg/kg (the ISA reaches 62 and 52%); the preparation introduced in doses of 50, 100, and 200 mg/kg did not exhibit an antileucotic effect.

Derivative IVa, similarly as III, is more toxic than its methylated analog IIIa; the antileucotic effect of IIIa is inconstant and fluctuates within 15-32%.

Thus, the alkylation of substituted 4-thio-6-azauracils and 6-azacytosines under the conditions of the simplified variant of the "silyl condensation" proceeds similarly to the glycosylation reaction of these compounds. The synthesized N^1-tetrahydrofuranyl-6-azacytosine and 5-methyl-6-azacytidine on a model leucosis strain exhibit an inhibiting effect which is somewhat lower than that of the 6-azacytosine riboside standard.

REFERENCES

1. N. A. Petrusha, V. A. Milinevskaya, and V. E. Popova, "Components of Nucleic Acids:" Summaries of Lectures of the 7th All-Union Symposium on a Directed Search for Medicinal Compounds [in Russian], Riga (1989), pp. 72-73.
2. S. A. Giller, M. Yu. Lidak, and R. A. Zhuk, et al., Khim. Geterotsikl. Soedin., No. 2, 375-377 (1969).
3. R. A. Zhuk, A. É. Berzina, L. A. Sherin', et al., Khim. Geterotsikl. Soedin., No. 9, 1258-1259 (1977).
4. A. S. Shalamai, S. S. Ognyanik, I. V. Alekseeva, and V. S. Goncharenko, "Method of preparation of 6-azacytidine," Inventor's Certificate 1,622,881 (USSR); Otkrytiya, No. 5 (1991).
5. I. V. Alekseeva, A. S. Shalamai, A. V. Turov, et al., Ukr. Khim., Zh., **44**, No. 10, 1064-1069 (1978).
6. J. Baranek and F. Sorm, Coll. Czechosl. Chem. Commun., **28**, No. 2, 469-479 (1963).
7. H. Hrebabesky and J. Baranek, Coll. Czechosl. Chem. Commun., **39**, No. 4, 976-981 (1974).
8. G. T. Frumin, Khim.-farm. Zh., **25**, No. 6, 15-18 (1991).