

ACYCLIC ANALOGS OF NUCLEOSIDES. SYNTHESIS AND IN VITRO ANTIVIRAL ACTIVITY OF HYDROXYALKYL-2-(TRIFLUOROMETHYLTHIOMETHYL)BENZIMIDAZOLES

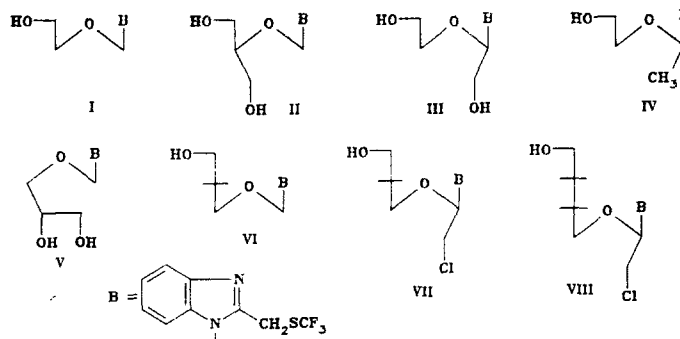
A. É. Yavorskii, A. V. Turov, I. V. Gogoman, A. I. Sobko, V. N. Tatskaya, V. G. Kvachev, and V. L. Florent'ev

UDC 547.785.5'412.122.07:615.281.3

In a search for novel antiviral compounds of the 'doubly modified' nucleoside type, we have prepared 1-(4-hydroxy-2-oxabutyl)-, 1-(4-hydroxy-3-hydroxymethyl-2-oxabutyl)-, 1-(4-hydroxy-1-hydroxymethyl-2-oxabutyl)-, 1-(4-hydroxy-1-methyl-2-oxabutyl), 1-(4,5-dihydroxy-2-oxapentyl)-, 1-(5-hydroxy-2-oxapentyl), 1-(5-hydroxy-1-chloromethyl-2-oxapentyl)-, and 1-(6-hydroxy-1-chloromethyl-2-oxahexyl)-2-(trifluoromethylthiomethyl)benzimidazole. They were obtained by condensing the trimethylsilyl derivative of 2-(trifluoromethylthiomethyl)benzimidazole with alkylating agents in the presence of an equimolar mixture of trifluoromethanesulfonic acid and trimethylchlorosilane. These nucleoside analogs showed moderate antiviral activity against some RNA viruses.

Continuing a study of structure-biological activity relationships in 'doubly-modified' nucleosides [1-3], we have obtained some hydroxyalkylated 2-(trifluoromethylthiomethyl)benzimidazoles, and examined their in vitro antiviral activity against some RNA entero- and coronaviruses.

2-(Trifluoromethylthiomethyl)benzimidazole (XXVI) was obtained by the Philips cyclization of o-phenylenediamine with (trifluoromethylthio)acetic acid.



The protected nucleoside analogs (XVIII-XXV) (Table 1) were obtained by condensing the trimethylsilyl derivative (IX) (without isolation) with the alkylating agents (X-XVII) in dry acetonitrile, in the presence of an equimolar mixture of trimethylchlorosilane and trifluoromethanesulfonic acid as catalyst. (Formula, next page, below Table 1.)

The acetyl groups in the protected derivatives were removed by treatment with methanolic ammonia, to give the unprotected nucleoside analogs (I-VIII) (Tables 1-3).

The PMR spectra of the unprotected nucleoside analogs in DMSO-D<sub>6</sub> showed particularly characteristic signals for the hydroxy-groups. For example, the single primary hydroxyl in the spectra of (VI-VIII) appears as a triplet of integral intensity 1H. In analogs (I-V), the signals for the hydroxy-groups overlap those for the CH<sub>2</sub>S methylene protons. The presence of hydroxy-groups in these compounds is shown by the decrease in the integral intensity of the signal at 4.70-4.75 ppm on treating the sample with D<sub>2</sub>O. The CH<sub>2</sub> signal in the spectra of (III-V), (VII), (VIII), (XX-XXII), (XXIV), and (XXV) was present as an AB quartet as a result of

Institute of Molecular Biology, Academy of Sciences of the USSR. Moscow 117984. Kiev State University, Kiev 252017. Ukrainian Veterinary Research Institute, Kiev 252000. Translated from Khimiya Geterotsiklicheskih Soedinenii, No. 4, pp. 493-496, April, 1989. Original article submitted August 3, 1987.

TABLE 1. Results of the Synthesis of Acyclic Nucleoside Analogs

Alkylating agent	Protected analog		Unprotected analog		
	compound	yield, %	compound	yield, %	mp, °C (from ethyl acetate-ether)
X	XVIII	61	I	75	69...70
XI	XIX	48	II	84	117...118
XII	XX	75	III	80	130...131
XIII	XXI	66	IV	85	105...106
XIV	XXII	50	V	90	87...88
XV	XXIII	60	VI	90	100...101
XVI	XXIV	60	VII	95	Oil
XVII	XXV	55	VIII	92	Oil

\*All compounds were obtained as oils.

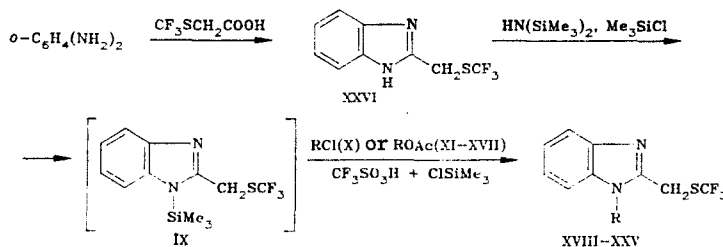
TABLE 2. PMR Spectra of Acyclic Analogs of 2-(Trifluoromethylthio)-benzimidazole Nucleosides

Compound	$\delta$ , ppm* (SSCC, Hz)			
	1'-H or 1'-CH <sub>3</sub>	2'-H	CH <sub>2</sub> CO <sub>2</sub> or OH	CH <sub>2</sub> SCF <sub>3</sub>
XVIII	5,66 s	—	2,02 s	4,49s
I	5,76 s	—	†	4,69s
XIX	5,73 s	—	1,94 s	4,55s
II	5,83 s	—	†	4,73s
XX	4,40 d.d (11,6; 5,2); 4,61 d.d (11,6; 6,3)	5,89 d.d (5,2; 6,3)	1,96 s	4,42d (13,9); 2,00 s 4,58d (13,9)
III	3,82 m	5,83 d.d (5,0; 5,5)	5,18 t ‡	4,70 br.s
XXI	1,80 d (6)	5,78 q (6)	1,99 s	4,49 br.s
IV	1,69 d (6)	6,04 q (6)	†	4,73 br.s
XXII	5,66 s	—	1,95 s	4,44 br.s
V	5,72 s	—	4,45 t ‡	4,68 br.s
XXIII	5,61 s	—	1,93 s	4,48 s
VI	5,71 s	—	4,41 t	4,66 s
XXIV	4,02 m	5,65 d.d (5,0; 6,0)	1,90 t	4,41 d (14) 4,64 d (14)
VII	4,13 m	6,08 t (6)	4,42 t	4,72 br.s
XXV	4,03 m	5,73 d.d (5,5; 6,0)	2,02 s	4,42 d(14,5); 4,64 d (14,5)
VIII	4,08 d.d (11; 6); 4,19 d.d (11; 6)	6,08 t (6)	4,37 t	4,72 br.s

\*The spectra of (I-VIII) were obtained in DMSO-D<sub>6</sub>, and of (XVIII-XXV) in CDCl<sub>3</sub>. Only the most characteristic signals are given; the signals for the benzene ring protons were seen as a multiplet at 7-8 ppm.

†The signal for the primary hydroxy-group overlapped the signal for CH<sub>2</sub>S.

‡The signal for the secondary hydroxy-group overlapped the signal for CH<sub>2</sub>S.



X, XVIII R=AcOCH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>-; XI, XIX R=AcOCH<sub>2</sub>CH(CH<sub>2</sub>OAc)OCH<sub>2</sub>-; XII, XX R=AcOCH<sub>2</sub>CH<sub>2</sub>OCH(CH<sub>2</sub>OAc)-; XIII, XXI R=AcOCH<sub>2</sub>CH<sub>2</sub>OCH(CH<sub>3</sub>)-; XIV, XXII R=AcOCH<sub>2</sub>CH(OBz)CH<sub>2</sub>OCH<sub>2</sub>-; XV, XXIII R=AcOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>-; XVI, XXIV R=AcOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OCH(CH<sub>2</sub>Cl)-; XVII, XXV R=AcOCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OCH(CH<sub>2</sub>Cl)-

the magnetic nonequivalence of the methylene protons (these protons are diastereotopic). The protons of the 1'-CH<sub>2</sub> group in III, V, VII, VIII, XX, XXII, XXIV and (XXV) are diastereotopic in exactly the same way. In compounds (III), (V), (XXI), and (XXII), however, the diastereotopic effect is so small that this signal is present as a broadened singlet, the outer peaks

TABLE 3. UV Spectra of Acyclic Nucleoside Analogs  
[2-(Trifluoromethylthiomethyl)benzimidazoles] in  
Methanol

Com- pound	$\lambda_{\max}$ , nm ( $\epsilon$ )	Com- pound	$\lambda_{\max}$ , nm ( $\epsilon$ )
XVIII	250 (7700); 277 (7140)	I	250 (7750); 277 (7170)
XIX	251 (7790); 277 (7000)	II	250 (6950); 277 (5800)
XX	250 (10510); 277 (10500)	III	250 (6950); 277 (5500)
XXI	250 (9750); 278 (9790)	IV	252 (7660); 277 (6670)
XXII	251 (7800); 277 (6500)	V	250 (8100); 277 (6670)
XXIII	250 (9530); 277 (9050)	VI	252 (9050); 277 (8900)
XXIV	252 (8200); 277 (6300)	VII	250 (8300); 277 (6450)
XXV	252 (7750); 277 (7700)	VIII	251 (7900); 277 (7750)

being masked by noise. Only in the spectra of VII, VIII, XX, XXIV and XXV is the 1'-CH<sub>2</sub> signal seen as the AB region of an ABX system (two doublets of doublets).

Biological testing showed that in concentrations of 100-200  $\mu\text{g}/\text{kg}$ , compounds (I-III) displayed antiviral activity against a coronavirus (the causative agent of transmissible swine gastroenteritis) and swine enterovirus, the known inhibitor of RNA-viruses 2-( $\alpha$ -hydroxybenzyl)-benzimidazole showing a protective effect at concentrations of up to 50  $\mu\text{g}/\text{ml}$ . Test for cytotoxicity showed that (I-III) had no effect on cell morphology at concentrations up to 200  $\mu\text{g}/\text{ml}$ .

#### EXPERIMENTAL

UV spectra were obtained on a Specord UV-VIS, and PMR spectra on a Bruker WP-100 SY spectrometer. TLC was carried out on Silufol UV-254 plates in the system chloroform-methanol (9:1). The sorbent used in column chromatography was silica gel L 40/100. The elemental analyses for all the compounds did not differ from the calculated values by more than 2%.

The synthesis of the alkylating agent (X) has been described in [4], (XI) and (XIV) in [5], (XII) in [6], and (XIII) and (XV-XVIII) in [3].

2-(Trifluoromethylthiomethyl)benzimidazole (XXVI). A mixture of 10.4 g (0.1 mole) of o-phenylenediamine and 16.4 g (0.1 mmole) of (trifluoromethylthio)-acetic acid [7] in 200 ml of HCl (4 moles/liter) was boiled for 6 h. The mixture was cooled, and neutralized with aqueous ammonia to pH 7. The product was filtered off, dried in vacuo, and recrystallized from benzene to give 16.2 g (70%) of product, mp 164-165°C.

Hydroxyalkyl Derivatives of 2-(Trifluoromethylthiomethyl)benzimidazole. To a suspension of 500 mg (2.155 mmole) of the base in 5 ml of hexamethyldisilazane was added 0.5 ml of trimethylchlorosilane, and the mixture boiled for one hour. The alkylating agent (2.2 mmole) was then added with cooling, followed by 0.28 ml (2.2 mmole) of trimethylchlorosilane, 0.19 ml (2.2 mmole) of trifluoromethanesulfonic acid, and 10 ml of dry acetonitrile. The mixture was kept for 24 h at 20°C, poured into 50 ml of saturated sodium bicarbonate solution, and extracted with chloroform (3  $\times$  50 ml). The combined extracts were dried over Na<sub>2</sub>SO<sub>4</sub>, and evaporated under reduced pressure. The residue was chromatographed on a 2  $\times$  10 cm column of silica gel, 40-100  $\mu\text{m}$ , and eluted with a 20% solution of hexane in chloroform. The resulting protected nucleoside was dissolved in half-saturated methanolic ammonia at 0°C, kept for 24 h at 20°C, and evaporated under reduced pressure. The residue was chromatographed on a 2  $\times$  6 cm column of silica gel with 5% hexane in chloroform. The resulting unprotected nucleoside was treated with dry ether in the cold, and recrystallized from ethyl acetate-ether (Table 1).

The antiviral activity of the compounds was assessed in a transplanted two-three day culture of swine embryo cells infected with reference standard Purdue transmissible swine gastroenteritis virus, 115th passage, (titer 6lg CDT<sub>50</sub>/ml) and a vaccine strain of swine enterovirus, Derbyshire (1982) stereotype, 70th passage (8lg CDT<sub>50</sub>/ml). Antiviral activity was measured by the inhibition of the cytopathic effects of the virus. The dose used was 1000 DCT<sub>50</sub> per ml. The test compounds were dissolved in the culture medium (5% blood hydrolyzate) to concentrations of 200, 100, 50, 10, 5, 1, and 0.2  $\mu\text{g}/\text{ml}$ . Controls were set up for the cytopathogenic effects of the viruses, the medium without addition of the test compounds, and the toxicity of the test compounds in the absence of viruses. The cytopathogenic effects of the viruses in the controls and in the presence of the test compounds were assessed 24, 48, 96, and 144 h following infection of the cell cultures.

## LITERATURE CITED

1. A. É. Yavorskii, A. V. Stetsenko, S. G. Zavgorodnii, and V. L. Florent'ev, *Khim. Geterotsikl. Soedin.*, No. 2, 198 (1988).
2. A. É. Yavorskii, I. V. Gogoman, V. N. Boiko, A. I. Sobko, V. N. Tatskaya, V. G. Kvachev, and V. L. Florent'ev, *Khim. Geterotsikl. Soedin.*, No. 5, 632 (1988).
3. A. É. Yavorskii, L. N. Reshot'ko, A. A. Kucheryavenko, and V. L. Florent'ev, *Khim.-farm. Zh.*, No. 7, 833 (1988).
4. L. Yu. Tychinskaya and V. L. Florent'ev, *Bioorg. Khim.*, 4, 1461 (1978).
5. T. L. Tsilevich, S. G. Zavgorodnii, U. Marks, L. V. Ionova, and V. L. Florent'ev, *Bioorg. Khim.*, 12, 819 (1986).
6. S. G. Zavgorodnii, E. V. Efimtseva, S. G. Mikhailov, T. L. Tsilevich, A. E. Yavorskii, and V. L. Florent'ev, *Khim. Geterotsikl. Soedin.*, No. 1, 91 (1988).
7. V. V. Orda, L. M. Yagupol'skii, and V. F. Bystrov, *Zh. Obshch. Khim.*, 35, 1628 (1965).