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SYNTHESIS AND ANTIVIRAL ACTIVITY OF DIHYDROXYCYCLOHEXYL PYRIMIDINE AND PURINE CARBOCYCLIC NUCLEOSIDES

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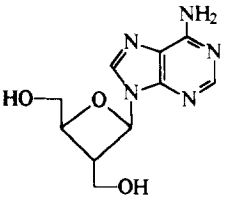
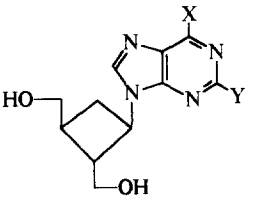
Abstract Some novel dihydroxycyclohexyl pyrimidine and purine carbocyclic nucleosides were prepared and tested for inhibitory activity against Cytomegalovirus and Herpes virus-1 and -2.

The pathologies related to viral and/or neoplastic affections are one of the primary causes of death in the world. Natural nucleosides have been the primary models for the design of the most important antitumoral and/or antiviral antimetabolites.[1,2] The difference which these last two classes of compounds exhibit compared with reference molecules concerns both the basic heterocyclic portion and the glucidic one. The broad majority of therapeutic nucleosides contain a glycosidic moiety. It has been found that its substitution with a carbocyclic

structure including several hydroxy groups leads to compounds sometimes possessing interesting pharmacological profiles.[3-8] The carbocyclic system most frequently used is the cyclopentane one, chosen because of its structural analogy with the tetrahydrofuran ring present in natural nucleosides.[9,10] More recently, the interesting pharmacological properties of a purine derivative of an oxetane (**oxetanocine A**, **1**) and of its cyclobutane carbanalogues (**cyclobut-A**, **2** or **cyclobut-B**, **3**) have been reported,[11] these compounds were found to be active towards herpes viruses and HIV, thus indicating that in carbocyclic nucleoside (carbonucleoside) analogues, the cyclic structure was not necessarily a cyclopentane.

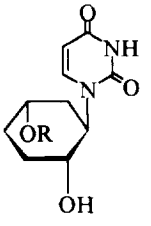


1



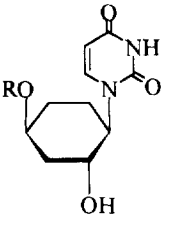
2, **X** = NH_2 , **Y** = **H**
3, **X** = **OH** , **Y** = NH_2

On the basis of these data, we decided to prepare some new carbonucleosides in which the carbocyclic structures, different from those already utilized, are linked to pyrimidine (Uracil) or purine (Adenine and Guanine) bases present in pharmacologically active nucleosides.

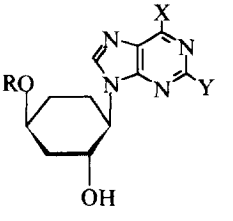


A

4, **R**= **Bn**
5, **R**= **H**



6, **R**= **Bn**
7, **R**= **H**



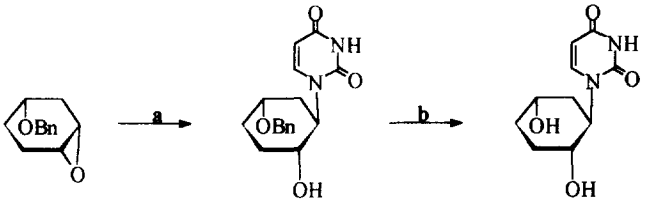
B

8, **R** = **Bn** **X** = NH_2 , **Y** = **H**
9, **R** = **H** **X** = NH_2 , **Y** = **H**
10, **R** = **Bn** **X** = **OH** , **Y** = NH_2
11, **R** = **H** **X** = **OH** , **Y** = NH_2

We therefore synthesized the new cyclohexanyl carbonucleosides[12] of type **A** (**4** and **5**) and **B** (**6-11**), which present, on the cyclohexane ring, a hydroxy group adjacent and trans with respect to the basic nucleus; this relationship is the same present in natural ribonucleosides and also in several synthetic ones. **A** and **B** differ in the position and stereochemistry of the other substituent on the cyclohexane ring.

As starting material for the synthesis of **4-11**, we used the cis **12** and trans **13** benzyloxyepoxides.[13] The reaction of the lithium salt of uracil with the cis epoxide **12** at 140 °C in the presence of $LiClO_4$ afforded only the trans regioisomer **4**[14, 15] which was submitted to hydrogenolysis with H_2 in the presence of 10% Pd/C to yield the corresponding diol derivative **5**[15] (Scheme 1).

SCHEME 1



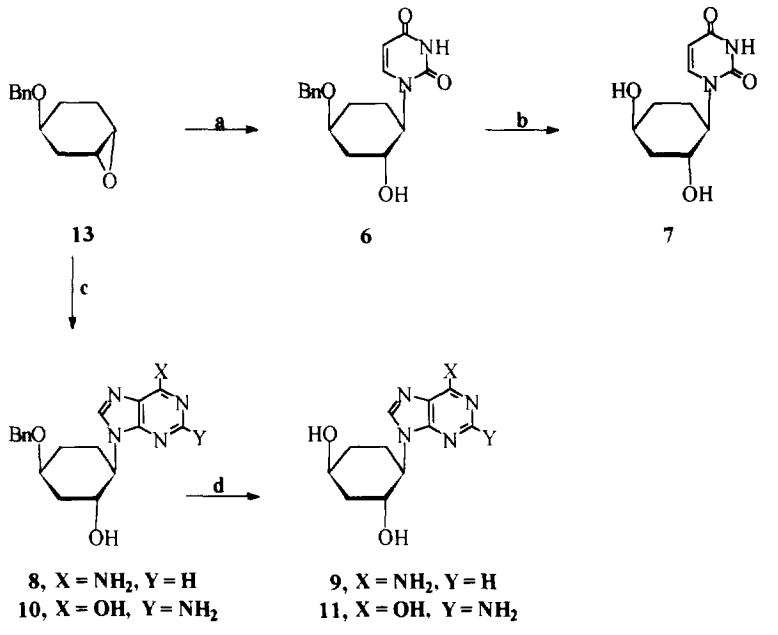
12 **4** **5**

Reagents and conditions: (a) Uracil, *n*-BuLi 1.6M hexane, $LiClO_4$, DMF, 140 °C 24 h, 30%; (b) H_2, Pd/C 10%, CH_3COOH cat., MeOH, 24 h, 65%.

The above-described synthetic conditions were also used for the preparation of the uracil carbocycles **6**[15] and **7**[15], starting in this case from the trans epoxide **13**. In the case of the synthesis of the purine derivatives **8-11**, the opening of **13** was carried out at 110 °C using the sodium salt of adenine for **8**[14,15] or the sodium salt of guanine for **10**[14,15], generated from the appropriate purinic base with NaH in DMF. Hydrogenolysis of benzyl derivatives **8** and **10** with H_2 in the presence of 10% Pd/C gave the desired carbonucleosides **9**[15] and **11**[15] (Scheme 2).

The antiviral activity of the compounds synthesized was evaluated by the plaque-reduction method against Cytomegalovirus (CMV) in PEU cells and Herpes virus 1 (HSV-1) and 2 (HSV-2), in Vero cells[17,18]. Results are shown in Table 1. The carbonucleosides **4**, **5** and **7** exhibited quite a good efficacy against the three virus species tested, while the carbonucleosides **8**, **9** and **10** were effective only against HSV-1 and -2. No cytotoxicity was observed for any of the compounds.

SCHEME 2



Reagents and conditions: (a) Uracil, *n*-BuLi 1.6 M hexane, $LiClO_4$, DMF, 140 °C, 24h, 34% H_2, Pd/C 10%, CH_3COOH cat., MeOH, 24 h, 65%; (c) Adenine or Guanine, NaH, DMF, 110 °C, 24 h, 20% for Aden. or 23% for Guan.; (d) H_2, Pd/C 10%, CH_3COOH, 48 h.

Table 1. Antiviral activity and cytotoxicity of the carbonucleosides.

Compound	EC_{50} (μM)[a]			IC_{50} (μM)[b]
	CMV	HSV-1	HSV-2	PEU cells
4	10.0	10.0	10.0	>250
5	0.97	10.0	0.97	>250
6	10.0	158	158	>250
7	10.0	0.97	0.97	>250
8	147	16.8	16.2	>250
9	200	28.5	30.0	>250
10	140	21.2	22.1	>250
11	188	188	188	>250

[a]Concentration required to inhibit CMV-, HSV-1- or HSV-2-induced cytopathic effects by 50 %.
[b]The lowest concentration of the compound producing overt cytotoxicity in virus-free cultures.

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12. Recently new nucleosides different from ours in which the glucidic portion has been substituted with a cyclohexanol nucleus have been reported in literature. See for example: [a] Ramesh,K.; Wolfe, M.S.; Lee, Y.; Velde, D.V.; Brochardt, R.T. *J. Org. Chem.* **1992**, *57*, 5861. [b] Jähne, G.; Winkler, I.; Helsberg, M.; Hänel, H. *European Patent Application* No. 92111063.1 filed June 30, 1992. [c] Shiozawa, A.; Matsubara K.; Nagata, T.; Hoshino, H.; Saki, J. *European Patent Application* No. 91111925.3 filed July 17, 1991. [d] Arango, J.H.; Geer, A.; Rodriguez, J.; Young, P.E.; Scheiner, P. *Nucleosides and Nucleotides* **1993**, *12*, 773.
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14. The trans relationship between the OH and the basic heterocyclic portion in the addition products (**4**, **6**, **8** and **10**) of epoxides **12** and **13** with pyrimidinic or purinic bases was assumed on the basis of the usual anti additions to these epoxide systems.[16] The regiochemistry of the addition products (**4**, **6**, **8** and **10**) was assumed on the basis of findings for ring opening reactions of **12** and **13** with nucleophiles.[13] However the exact structure and regiochemistry of the benzyl derivative **4** was unequivocally determined by X-ray analysis (material available from our laboratory). The site of alkylation (N-9) for the purine derivatives (**8-11**) was determined by UV spectroscopy: data obtained for **9** and **11**[15] were in close agreement with those found for the natural purinic nucleosides.

15. All compounds were well characterized spectroscopically. **4**: m.p. 237 °C; ^{1}HNMR (200 MHz, DMSO-d_6) δ 11.13 (s, 1H), 7.69 (d, J=8.0 Hz, 1H), 7.22-7.36 (m, 5H), 5.54 (d, J=8.0 Hz, 1H), 4.92 (m, 1H), 4.39-4.51 (m, 3H), 3.62-3.74 (br, 2H), 1.37-2.01 (m, 6H). **5**: m.p. 247 °C; ^{1}HNMR (200 MHz, DMSO-d_6) δ 11.09 (s, 1H), 7.66 (d, J=8.0 Hz, 1H), 5.51 (d, J=8.0 Hz, 1H), 4.84 (m, 1H), 4.42-4.62 (m, 2H), 3.88-3.97 (m, 1H), 3.61 (br, 1H), 1.44-1.67 (m, 6H). **6**: m.p. 189 °C; ^{1}HNMR (200 MHz, DMSO-d_6) δ 11.15 (s, 1H), 7.54 (d, J=8.0 Hz, 1H), 7.23-7.38 (m, 5H), 5.52 (d, J=8.0 Hz, 1H), 4.91 (m, 1H), 4.40-4.53 (m, 2H), 3.91-4.09 (m, 2H), 3.75 (s, 1H), 1.37-2.24 (m, 6H). **7**: m.p. 189 °C; ^{1}HNMR (200 MHz, DMSO-d_6) δ 11.13 (s, 1H), 7.56 (d, J=8.0 Hz, 1H), 5.53 (d, J= 8.0 Hz, 1H), 4.81 (m, 1H), 4.55 (m, 1H) 3.86-4.07 (m, 3H), 1.33-2.03 (m, 6H). **8**: m.p. 262 °C; ^{1}HNMR (200 MHz, DMSO-d_6) δ 8.10 (s, 2H), 7.24-7.45 (m, 5H), 7.12 (s, 2H), 4.83 (m, 1H), 4.45-4.58 (m, 2H), 4.28-4.40 (m, 1H), 4.07-4.20 (m, 1H), 3.82 (br, 1H), 1.43-2.45 (m, 6H). **9**: m.p. 258 °C; ^{1}HNMR (200 MHz, DMSO-d_6) δ 8.08 (s, 2H), 7.10 (s, 2H), 4.73 (m, 1H), 4.61 (br, 1H), 4.28-4.36 (m, 1H), 4.01-4.14 (m, 2H), 1.39-2.43 (m, 6H); UV λ_{max} (MeOH) sh 261 nm (ε 13300). **10**: m.p. 295 °C dec.; ^{1}HNMR (200 MHz, DMSO-d_6) δ 10.52 (brs, 1H), 7.71 (s, 1H), 7.25-7.38 (m, 5H), 6.34 (s, 2H), 4.87 (m, 1H), 4.44-4.57 (m, 2H), 3.97-4.17 (m, 2H), 3.79 (br, 1H), 1.39-2.28 (m, 6H). **11**: m.p. 290°C dec.; ^{1}HNMR (200 MHz, DMSO-d_6) δ 10.56 (brs, 1H), 7.67 (s, 1H), 6.54 (s, 2H), 4.76 (br, 1H), 4.12-4.16 (m, 1H), 3.85-3.99 (m, 3H), 1.34-2.12 (m, 6H); UV λ_{max} (MeOH) sh 254 nm (ε 12280).

16. See for example : Gorzynski Smith, J. *Synthesis*, **1984**, 629.

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