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Synthesis and biological evaluation of novel homochiral carbocyclic nucleosides from 1-amino-2-indanols

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ABSTRACT

New chiral purinyl and 8-azapurinyl carbanucleoside derivatives based on indanol were synthesized from commercial available (1*S*,2*S*)-*trans*-1-amino-2-indanol and (1*R*,2*R*)-*trans*-1-amino-2-indanol using a linear methodology. The antiviral activity and cytotoxicity of these compounds were evaluated against herpes simplex virus type 1 (HSV-1) in Vero cells, bovine viral diarrhea virus (BVDV) in Mardin-Darby bovine kidney (MDBK) cells and hepatitis B virus (HBV) in HepG2 2.2.15 cell line. Three compounds, showed an inhibition of the HBsAg levels similar to reference drug lamivudine. One chloropurinyl nucleoside, derived from the *cis*-1-amino-2-indanol, was cytotoxic on MDBK cells and it could be a lead for developing anticancer agents.

1. Introduction

Carbocyclic nucleosides, where the furanose oxygen atom of the normal nucleosides is replaced by a methylene group, have received extraordinary attention over the last two decades. As is well known this modification makes carbocyclic nucleosides more resistant to hydrolases than the natural nucleosides themselves, but does not prevent their enzymatic conversion to nucleotide analogues. As a result, they can possess antiviral, antineoplastic activity or other potentially biological properties.[1–4]

Between the different carbanucleosides prepared to date, (−)-carbovir synthesized for the first time by Vince's group in 1988[5] has shown significant anti-HIV activity, similar to AZT, through the selective inhibition of HIV-1 reverse transcriptase (HIV-RT). However, (−)-carbovir was removed from clinical trial test due to its pharmacokinetic problems. The antiviral potency of (−)-carbovir triggered an explosive synthetic effort of preparation of carbanucleoside derivatives, such as (−)-abacavir. The success of abacavir has been attributed in part to the rigidity of the molecule afforded by its double bond between C-2 and C-3[6,7] and in part to its lipophilicity that increase the ability to penetrate into the central nervous system, an important reservoir of the HIV and other viruses (Fig. 1).

Inspired on that, several research groups have synthesized and investigated the biological properties of additional carbovir analogues such as, (+)-carbovir,[8] (−) and (+)-5′-norcarbovir, their corresponding triphosphates,[9] and *trans*-carbovir[10] (Fig. 2).

(+)-Carbovir was found to be less active as an anti-HIV agent than (−)-carbovir in vitro test. The triphosphate of (−)-5′-norcarbovir showed good activity as an inhibitor of HIV-1 RT similar to the triphosphate of (−)-carbovir. Surprisingly, compound (+)-5′-norcarbovir showed even greater activity as an inhibitor of HIV-1 RT. Biological activity has not yet been described for *trans*-carbovir.[10]

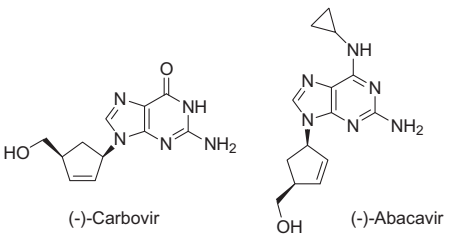

Figure 1. Bioactive carbanucleosides.

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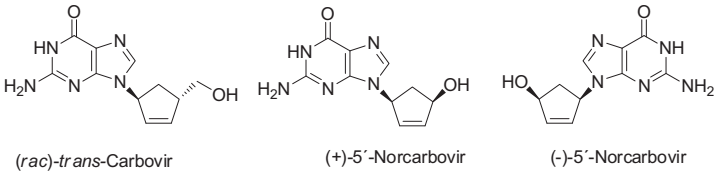


Figure 2. Nucleosides derivated from (−)-carbovir.

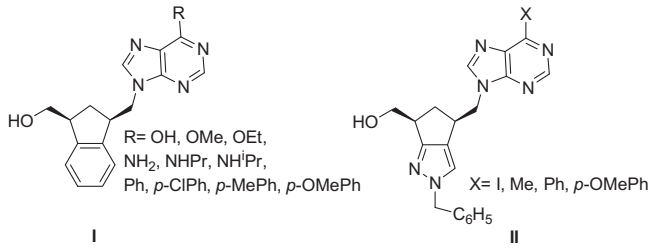


Figure 3. Carbanucleosides with an aromatic ring.

From 1990 several research groups have been investigating the biological properties of others carbanucleosides, such as **I** and **II**, in which the planar region of the double bond, present in carbovir and abacavir, was replaced by a benzene ring[11–13] (**I**) or an aromatic heterocycle[14–17] (**II**), seeking to modify the lipophilicity and polar interactions of this region of the molecule while preserving the rigidity (Fig. 3). Most of the new compounds showed little if any antiviral activity against HIV, but in a broader antiviral survey, some of them, proved to be active against human cytomegalovirus and varicella zoster virus at subcytotoxical concentrations.[15]

In the last decade several research groups have prepared different series of carbanucleosides derived from indane and these bear purine and pyrimidine bases and are of the type 1′-homoderivative[18] and 5′-nor-1′-homoderivative,[19] but in all published examples the indanic core has a substitution *cis*-1,3. The increased development of single-enantiomer pharmaceuticals and the interest in asymmetric synthesis has enhanced the need for convenient methods for the preparation of enantiopure materials.[20] In the search of easily accessible chiral templates for asymmetric transformations, chiral amino alcohols were generally identified as versatile reagents for the generation of enantiopure compounds. Amino alcohols that are not derived from the chiral pool sometimes offer additional advantages in terms of structural diversity and conformational properties. Among those, *cis*-1-amino-2-indanol[21] played a crucial role in the development of active pharmaceutical ingredients such as indinavir, a valuable HIV-1 protease inhibitor (PI) ligand and orally bioavailable aggrecanase inhibitors (Fig. 4).

Recently we have published[22] a short and efficient synthesis, based on a well-known methodology, of new homochiral purinyl and 8-azapurinyl carbanucleosides based on 1-amino-2-indanol. All of them were synthesized from the commercially available (1*R*,2*S*)-*cis*-1-amino-2-indanol and (1*S*,2*R*)-*cis*-1-amino-2-indanol. Herein, we report the synthesis of new indane carbanucleosides from *trans*- and *cis*-1-amino-2-indanol. We also report their cytotoxic activity against HepG2.2.15, Vero and MDBK cell lines and the antiviral activity against HSV-1, BVDV and HBV.

2. Results and discussion

2.1. Chemistry

In connection with our interest for the synthesis of mimetic carbanucleosides in which the sugar unit is replaced by an indane core, we have here devised a synthetic route towards enantiomerically pure indane carbanucleosides of 6-substituted purine and 8-azapurine **A** and **B**, based in the commercially available (1*R*,2*R*)- and (1*S*,2*S*)-*trans*-1-amino-2-indanols **1** and **2**, as chiral starting materials (Fig. 5). These compounds have not been described so far in the chemistry of nucleoside analogs.

Carbanucleosides were synthesized by a linear methodology from the amine group of enantiomerically pure amino alcohol **1** and **2**, following a classical approach to these compounds[13,23] (Scheme 1). Condensation of **1** with 5-amino-4,6-dichloropyrimidine in refluxing *n*-butanol containing triethylamine afforded **3**. Then to form the imidazole ring of the purinyl analogues, compound **3** was treated with triethylorthoformate in hydrochloric acid giving compound **4**. A triazole ring was also formed from **3** by intramolecular reaction of the diazonium salt of the primary amine group with sodium nitrite in an acidic medium, giving a highly unstable compound **5** (not isolated). This compound was converted in the 8-aza purine-6-hydroxyl derivative **6** or the 6-amino derivative **7** by treatment with 0.25 N sodium hydroxide or ammonium hydroxide, respectively.

For the preparation of enantiomeric nucleoside analogues (**9–12**) the same sequence of reactions was used starting from (1*S*,2*S*)-*trans*-1-amino-2-indanol **2** (Scheme 1).

In order to get the four diasteroisomers of compounds **7** and **12**, starting from (1*R*,2*S*)-*cis*-1-amino-2-indanol **13** and (1*S*,2*R*)-*cis*-1-amino-2-indanol **14**, and applying the same methodology, we synthesized diastereoisomeric carbanucleosides **15** and **16** (Scheme 2).

We also included, in the activity assays, the previously described compounds **17–20**[22], derived from the two enantiomers of the *cis*-1-amino-2-indanol (Fig. 6).

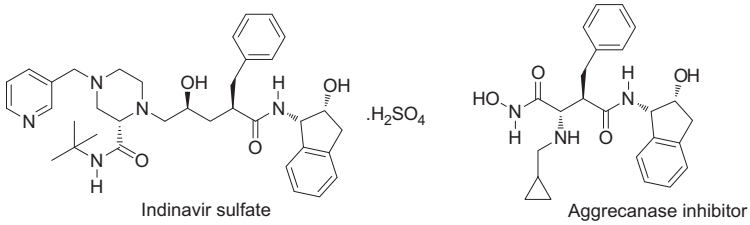


Figure 4. HIV-1 protease and aggrecanase inhibitors.

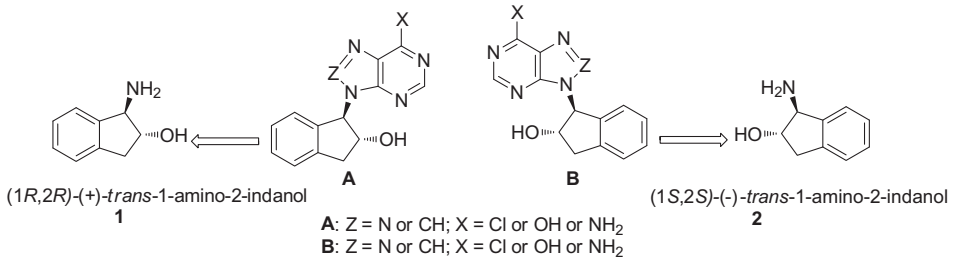
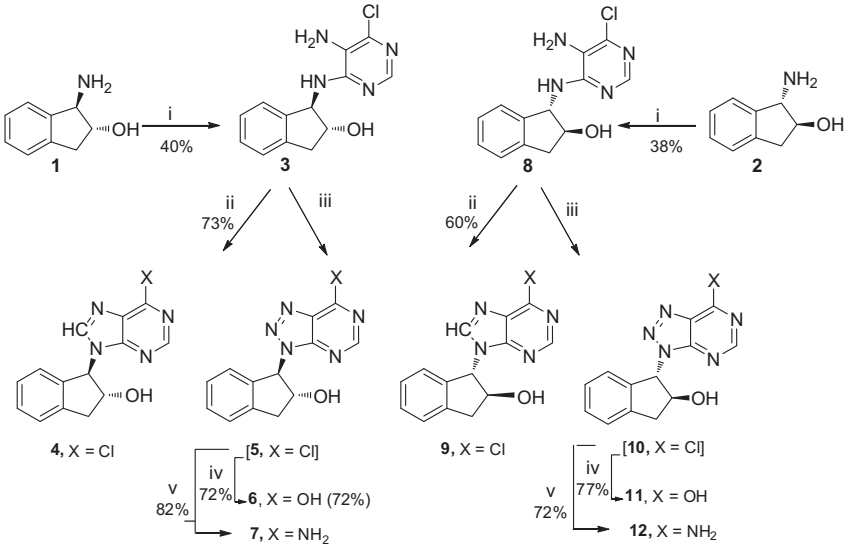
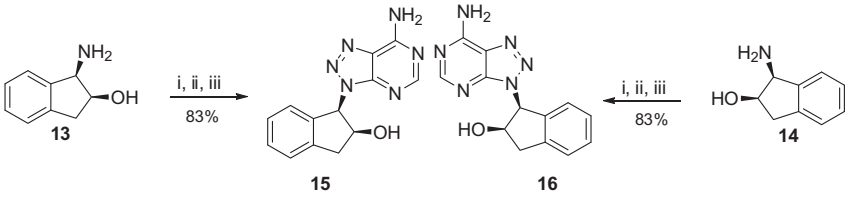


Figure 5. Retrosynthetic analysis to indanyl nucleosides.



Scheme 1. Reagents and conditions: (i) 5-amino-4,6-dichloropyrimidine, $(CH_3CH_2)_3N$, *n*-butanol, reflux, 24 h; (ii) triethylorthoformate, 12 N HCl, rt, 36 h; (iii)1 N HCl, $NaNO_2$, H_2O; (iv) 0.25 N NaOH, reflux, 6 h; (v) NH_4OH, reflux, 5 min.



Scheme 2. Reagents and conditions: (i) 5-amino-4,6-dichloropyrimidine, $(CH_3CH_2)_3N$, *n*-butanol, reflux, 24 h; (ii)1 N HCl, $NaNO_2$, H_2O; (iii) NH_4OH, reflux, 5 min.

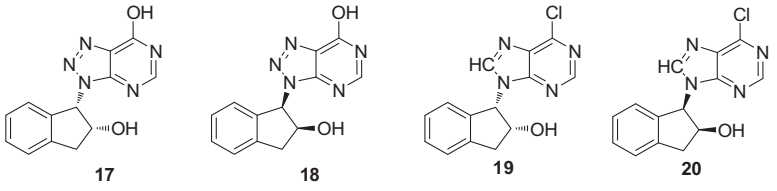


Figure 6. *cis*-Indanyl carbanucleosides.

2.2. Biological results

Once the 12 indane carbanucleosides were in hand, they were evaluated for their potential cytotoxic activity and their inhibitory activity against three selected viruses, such as representative of the different virus families, which diverse genome nature, viral polymerases and replication strategies.

The cytotoxicity against HepG2.2.15, Vero and MDBK cell lines were studied for homochiral indane carbanucleosides **4**, **6**, **7**, **9**, **11**, **12**, **15**, **16** and the previously described compounds **17–20**. These compounds showed low cytotoxicity (CC_{50} >500 μM) for the three cell lines tested, with the exception of the compound **19**. This compound was cytotoxic against MDBK cell line (CC_{50} = 14.31 μM) (Fig. 7).

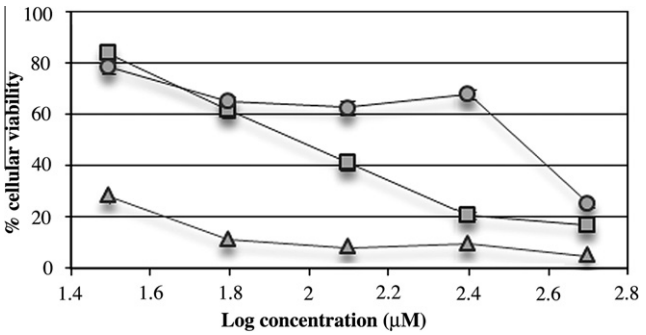


Figure 7. Cytotoxicity of compound **19** on MDBK (—▲—), Vero (—■—) or HepG2 2.2.15 (—◆—) cells.

Subconfluent monolayers of MDBK, Vero or HepG2 2.2.15 cells were incubated in presence or absence of two fold dilutions of **19** compound. After 72 h of incubation the cellular viability was evaluated by MTS/PMS method.

The evaluation of the antiviral activity of the 12 indane carbanucleosides demonstrated that these compounds exerted low inhibition values against BVDV and HSV-1 viruses (Table 1). However, against HBV several of these compounds showed some degree of inhibition of the HBsAg levels.

3. Conclusion

Here we report in detail, a convenient synthetic procedure for the preparation of novel purinyl- and 8-azapurinyl-carbanucleoside derivatives **4–7** and their enantiomers **9–12**, and diasteroisomers **15** and **16** obtained from the commercially available enantiomers of *trans*- and *cis*-1-amino-2-indanol as examples of a template in which the double bond of the cyclopentenyl nucleosides is incorporated in a benzene ring and a first characterization of their biological activities. Evaluation of the antiviral activity of these compounds showed that: (a) the *trans*-6-amino-8-azapurynil derivatives **7**, **12** and the *cis*-6-amino-8-azapurynil derivative **16** have a similar percentage of reduction of the HBsAg levels in HepG2 2.2.15 cells regardless of configuration of the starting 1-amino-2-indanol; (b) compound **16** showed a moderate inhibition on BVDV; (c) the cytotoxicity observed for compound **19** makes it as a potential lead compound for developing new cytotoxic agents.

4. Experimental section

4.1. Synthetic methods

4.1.1. General

Melting points were determined on a Thomas Hoover apparatus. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker AMX-500 and Bruker 300 spectrometers. High resolution mass spectrum (HRMS) was recorded on a micrOTOF-Q mass spectrophotometer. Optical rotations were measured with a Perkin–Elmer 141 polarimeter at 23 °C. IR spectra of samples, in KBr disk (solids), were recorded on a Perkin–Elmer Spectrum 1 FTIR spectrophotometer. Preparative thin layer chromatography (p-TLC) and thin layer chromatography analysis (TLC) were performed on Kieselgel 60 F_{254} (Merck) plates. The reagents were purchased from Aldrich and used without purification. (1*R*,2*R*)-(−)-*trans*-1-amino-2-indanol $[\alpha]_D^{23}$ −23.0 (*c* 1.0, ethanol); (1*S*,2*S*)-(+)-*trans*-1-amino-2-indanol $[\alpha]_D^{25}$ +23.0 (*c* 1.0, ethanol), both with 97% of ee by GLC. (1*R*,2*S*)-(+)-*cis*-1-amino-2-indanol $[\alpha]_D^{23}$ +63.0 (*c* 0.2, chloroform); (1*S*,2*R*)-(−)-*cis*-1-amino-2-indanol $[\alpha]_D^{22}$ −61.0 (*c* 0.5, chloroform), both with 99% of ee by GLC.

4.1.1.1. (1*R*,2*R*)-1-(5-amino-6-chloropyrimidin-4-ylamino)-2,3-dihydro-1*H*-inden-2-ol (3). A mixture of **1** (200 mg, 1.34 mmol) and 5-amino-4,6-dichloropyrimidine (220 mg, 1.34 mmol) in dry triethylamine (1.5 mL) and *n*-butanol (5.3 mL) was heated at reflux during 24 h under argon atmosphere. Then, the reaction mixture was cooled and the solvent removed under reduced pressure until dryness. The residue was suspended in EtOAc (5 mL) and was washed with water (2 × 5 mL). The organic layer was extracted with 10% HCl (3 × 10 mL). The aqueous layer was brought to alkaline pH (pH 8). Then, the solution was extracted with EtOAc (2 × 5 mL), dried on anhydrous Na_2SO_4 and the solvent was evaporated under vacuum. The solid obtained was purified by p-TLC (eluant EtOAc:Cl_2CH_2, 2:1) to afford compound **3** as a white solid (150 mg, 40%); 172–174 °C; $[\alpha]_D^{23}$ +48.1 (*c* 0.097, MeOH); IR: 3443, 3362, 3200, 2912, 1622 cm^{-1}; ^{1}H NMR (500 MHz) δ (ppm): 2.95 (dd, *J* = 7.3 Hz, *J* = 15.6 Hz, 1H, C*H*H), 3.37 (dd, *J* = 7.3 Hz, *J* = 15.8 Hz, 1H, C*H*H), 5.05 (c, *J* = 7.3 Hz, 1H, C*H*OH), 6.05 (d, *J* = 6.6 Hz, 1H, C*H*NH), 6.80 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.12 (t, *J* = 7.5 Hz, 1H, Ar*H*), 7.27 (t, *J* = 7.3 Hz, 1H, Ar*H*), 7.32 (d, *J* = 7.5 Hz, 1H, Ar*H*), 8.20 (s, 1H, N=C*H*–N); ^{13}C NMR (125 MHz) δ (ppm): 39.4, 69.3, 77.3, 123.8, 125.4, 127.4, 128.9, 130.0, 138.5, 140.3, 149.5, 150.8, 156.8. HRMS calcd for $C_{13}H_{13}ClN_4O$ *m*/*z* 277.0851, found 277.0859.

Table 1
Cytotoxic and antiviral activities of indane carbanucleosides.

Indanyl nucleoside	Vero CC_{50} (μM)	HSV-1 % I	MDBK CC_{50} (μM)	BVDV % I	HepG2 2.2.15. CC_{50} (μM)	HBsAg % I
6	>500	—	>500	—	>500	16.6 ± 4.9
11	>500	—	>500	—	>500	25.2 ± 0.8
17	>500	15.8 ± 6.3	>500	—	>500	27.7 ± 2.4
18	>500	12.8 ± 6.3	>500	—	>500	35.3 ± 0.7
7	>500	7.8 ± 1.2	>500	—	>500	42.1 ± 1.0
12	310.8	14.5 ± 2.0	>500	—	>500	37.2 ± 5.2
15	202.4	—	>500	19.4 ± 6.9	>500	17.6 ± 3.5
16	>500	20.9 ± 9.4	>500	42.1 ± 1.4	>500	41.4 ± 0.6
4	>500	7.2 ± 5.2	>500	—	>500	22.6 ± 0.2
9	>500	16.2 ± 4.3	>500	—	>500	14.2 ± 1.0
19	253.7	20.9 ± 7.3[a]	14.31	—[b]	308.5 ± 18.7	ND
20	>500	23.2 ± 1.0	>500	19.7 ± 0.9	>500	30.8 ± 2.0

CC_{50}: cytotoxic concentration 50: concentration that reduces cell viability by 50% respect to control cells; % I: percentage of reduction of number viral plaques (HSV-1) or viral cytopathic effect (BVDV) or HBsAg level (HBV) treated with each compound at 100 μM respect to virus control without drug, except for the compound **19**.
[a,b] Was assayed at $MNCC_{Vero}$ = 31.25 μM against HSV-1 and at $MNCC_{MDBK}$ = 2 μM against BVDV. Positive controls: lamivudine (3TC) at 20 μM = 45% of I of HBsAg; ribavirin against BVDV: EC_{50} = 4.6 μM and acyclovir against HSV-1: EC_{50} = 0.5 μM. ND = not determined. MNCC = maximal noncytotoxical concentration.

4.1.1.2. (1*S*,2*S*)-1-(5-amino-6-chloropyrimidin-4-ylamino)-2,3-dihydro-1*H*-inden-2-ol (8). Compound **2** (200 mg, 1.34 mmol) was converted into **8** (140 mg; 38%); $[\alpha]_D^{23}$ −47.5 (*c* 0.103, MeOH); according to the procedure described in Section 4.1.1.1. The IR, ^{1}H and ^{13}C NMR spectroscopic data were in good agreement with those described above for its enantiomer (+) **3**.

4.1.1.3. (1*R*,2*R*)-1-(6-chloro-9*H*-purin-9-yl)-2,3-dihydro-1*H*-inden-2-ol (4). A mixture of **3** (80 mg, 0.29 mmol), triethylorthoformate (1.8 mL) and 12 N HCl (0.1 mL) was stirred at room temperature for 36 h. Then, the solvent was removed under reduced pressure until dryness, and the residue was purified by p-TLC (eluant EtOAc) to afford chloropurine **4** as a white solid (60 mg, 73%); mp: >250 °C; $[\alpha]_D^{23}$ +133.1 (*c* 0.056, MeOH); IR: 3653, 3340, 3098, 1590, 1570 cm^{-1}; ^{1}H NMR (500 MHz) δ (ppm): 2.97 (dd, *J* = 1.8 Hz, 16.5 Hz, 1H, CH*H*), 3.23 (dd, *J* = 5.8 Hz, *J* = 16.3 Hz, 1H, C*H*H), 4.56 (td, *J* = 2.0 Hz, *J* = 5.26 Hz, 1H, C*H*OH), 5.05 (s, 1H, O*H*), 5.95 (d, *J* = 5.3 Hz, 1H, C*H*N), 6.95 (d, *J* = 7.6 Hz, 1H, Ar*H*), 7.29 (t, *J* = 7.6 Hz, 1H, Ar*H*), 7.35 (t, *J* = 7.3 Hz, 1H, Ar*H*), 7.35 (d, *J* = 7.3, 1H, Ar*H*), 7.39 (s, 1H, =N–C*H*=N), 7.41 (1H, s, N=C*H*–N); ^{13}C NMR (125 MHz) δ (ppm): 40.2, 62.1, 69.9, 125.3, 126.1, 127.8, 129.5, 131.8, 136.7, 141.2, 145.9, 150.4, 152.3, 154.7. HRMS calcd for $C_{14}H_{12}ClN_4O$ *m*/*z* 287.0694, found 287.0699.

4.1.1.4. (1*S*,2*S*)-1-(6-chloro-9*H*-purin-9-yl)-2,3-dihydro-1*H*-inden-2-ol (9). Compound **8** (80 mg, 0.29 mmol) was converted into **9** (50 mg, 60%); $[\alpha]_D^{23}$ −133.7 (*c* 0.050, MeOH); according to the procedure described in Section 4.1.1.3. The IR, ^{1}H and ^{13}C NMR spectroscopic data were in good agreement with those described above for its enantiomer (+) **4**.

4.1.1.5. 3-((1*R*,2*R*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-one (6). A cooled solution (0 °C) of aminochloropyrimidine **3** (100 mg, 0.36 mmol) in 1 N HCl (1.3 mL) was treated with a solution of sodium nitrite (36 mg, 0.48 mmol) in water (3.3 mL). The mixture was stirred and allowed to warm up to room temperature, refluxed for 1 h, and then, the solvent was removed under reduced pressure until dryness. The solid residue was purified by p-TLC (eluant EtOAc) to afford 8-azapurinone **6** (70 mg,72%), mp: 178–180 °C; $[\alpha]_D^{23}$ −22.8 (*c* 0.060, MeOH); IR: 3451, 3069, 2881, 1709, 1633 cm^{-1}; ^{1}H NMR (500 MHz) δ (ppm): 2.97 (dd, *J* = 7.6 Hz, *J* = 15.6 Hz, 1H, CH*H*), 3.40 (dd, *J* = 7.3 Hz, *J* = 15.8 Hz, 1H, CH*H*), 5.02 (c, *J* = 7.33, 1H, C*H*OH), 5.90 (br s, 1H, O*H*), 6.05 (d, *J* = 6.6 Hz, 1H, C*H*N),6.80 (d, *J* = 7.8 Hz, 1H, Ar*H*), 7.14 (t, *J* = 7.6, 1H, Ar*H*), 7.28 (t, *J* = 7.3, 1H, Ar*H*), 7.32 (d, *J* = 7.1, 1H, Ar*H*), 8.20 (s, 1H, N=C*H*–NH), 12.30 (br s, 1H, N*H*CO); ^{13}C NMR (125 MHz) δ (ppm): 39.7, 65.1, 71.7, 125.1, 125.9, 127.8, 128.3, 129.7, 139.0, 143.3, 149.7, 150.0, 157.4. HRMS calcd for $C_{13}H_{11}N_5NaO_2$ *m*/*z* 292.0805, found 292.0807.

4.1.1.6. 3-((1*S*,2*S*)-2-hydroxy-2,3-dihydro-1*H*-inden-1-yl)-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-7(6*H*)-one (11). Compound **8** (100 mg, 0.36 mmol) was converted into **11** (75 mg, 77%); $[\alpha]_D^{23}$ +23.9 (*c* 0.055, MeOH) according to the procedure described in Section 4.1.1.5. The IR, ^{1}H and ^{13}C NMR spectroscopic data were in good agreement with those described above for its enantiomer (−) **6**.

4.1.1.7. (1*R*,2*R*)-1-(7-amino-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl)-2,3-dihydro-1*H*-inden-2-ol (7). To a cooled (0 °C) solution of **3** (100 mg, 0.36 mmol) and 1 N HCl (1 mL), was added a solution of sodium nitrite (33 mg, 0.48 mmol) in water (3.7 mL). The mixture was stirred at 0 °C for 15 min, and then concentrated NH_4OH (2 mL) was added and heated under reflux during 5 min. The solvent was evaporated at reduced pressure, and the solid residue was purified by p-TLC (eluant EtOAc), to afford compound **7** (80 mg, 82%); mp: >280 °C; $[\alpha]_D^{23}$ −87.8 (*c* 0.077, MeOH); IR: 3503, 3019, 2847, 1722, 1694 cm^{-1}; ^{1}H NMR (500 MHz) δ (ppm): 2.76 (dd, *J* = 3.7 Hz, *J* = 16.0 Hz, 1H, CH*H*), 2.90 (dd, *J* = 5.5 Hz, *J* = 15.8 Hz, 1H, CH*H*), 4.24 (c, *J* = 5.0 Hz, 1H, C*H*OH), 4.76 (d, *J* = 4.8 Hz, 1H, O*H*), 6.07 (d, *J* = 5.3 Hz, 1H, C*H*N), 7.12–7.33 (m, 4H, Ar*H*), 8.26 (s, 1H, N=C*H*–N); ^{13}C NMR (125 MHz) δ (ppm): 40.8, 62.8, 72.1, 124.4, 125.1, 126.3, 129.0, 129.7, 138.1, 140.9, 146.3, 153.6, 158.8. HRMS calcd for $C_{13}H_{12}N_6NaO_2$ *m*/*z* 291.0970, found 291.0965.

4.1.1.8. (1*S*,2*S*)-1-(7-amino-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl)-2,3-dihydro-1*H*-inden-2-ol (12). Compound **8** (100 mg, 0.36 mmol) was converted into **12** (70 mg, 72%); $[\alpha]_D^{23}$ +89.1 (*c* 0.060, MeOH) according to the procedure described in Section 4.1.1.7. The IR, ^{1}H and ^{13}C NMR spectroscopic data were in good agreement with those described above for its enantiomer (−) **7**.

4.1.1.9. (1*S*,2*R*)-1-(7-amino-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl)-2,3-dihydro-1*H*-inden-2-ol (15). Compound **13** (100 mg, 0.36 mmol) was converted into **15** (80 mg, 83%), mp: >290 °C; $[\alpha]_D^{23}$ −35.4 (*c* 0.097, MeOH) according to the reported procedure in Section 4.1.1.7. IR: 3477, 3005, 2853, 1709, 1689 cm^{-1}; ^{1}H NMR (500 MHz) δ (ppm): 3.18 (dd, *J* = 6.9 Hz, *J* = 15.6 Hz, 1H, CH*H*), 3.25 (dd, *J* = 7.1 Hz, *J* = 15.8 Hz, 1H, CH*H*), 4.81 (m, 1H, C*H*OH), 5.27 (d, *J* = 5.5 Hz, 1H, O*H*), 6.23 (d, *J* = 6.6 Hz, 1H, C*H*N), 7.20–7.27 (m, 2H, Ar*H*), 7.35–7.40 (m, 2H, Ar*H*), 8.40 (s, 1H, N=C*H*–N); ^{13}C NMR (125 MHz) δ (ppm): 41.8, 63.8, 72.3, 125.4, 125.5, 127.4, 129.2, 129.6, 137.1, 140.4, 145.3, 152.6, 157.9. HRMS calcd for $C_{14}H_{17}N_6O_2$ (M+CH_3OH+H^+) found 301.1402 *m*/*z* 301.1407

4.1.1.10. (1*R*,2*S*)-1-(7-amino-3*H*-[1,2,3]triazolo[4,5-*d*]pyrimidin-3-yl)-2,3-dihydro-1*H*-inden-2-ol (16). Compound **14** (50 mg, 0.18 mmol) was converted into **16** (40 mg, 83%); $[\alpha]_D^{23}$ +34.5 (*c* 0.113, MeOH) according to the procedure described in Section 4.1.1.9. The IR, ^{1}H and ^{13}C NMR spectroscopic data were in good agreement with those described above for its enantiomer (−) **15**.

4.2. Cytotoxic and antiviral assays

4.2.1. Cells and viruses

4.2.1.1. Cells. African green monkey kidney (Vero, ATCC® number CCL-81™) and MDBK (ATCC® number CCL-22™) cells were grown and maintained in E-MEM (Eagle's Minimum Essential Media, GIBCO) supplemented with penicillin G 100 IU/mL, streptomycin 100 μg/mL and 10% fetal bovine serum (FBS, PAA) for Vero cells or 10% gamma irradiated fetal bovine serum (FBS gamma irradiated, PAA) for MDBK cells at 37 °C under 5% CO_2.

HepG2 2.2.15 human hepatoblastoma cell line, which constitutively produce HBV particles,[24] was grown and maintained with DMEM (Dulbecco's Modified Eagle Media, GIBCO) supplemented with 10% fetal bovine serum (FBS, GIBCO), penicillin G 100 IU/mL, streptomycin 100 μg/mL and G418 200 μg/mL at 37 °C under 5% CO_2.

4.2.1.2. Viruses. Herpes simplex virus type I, strain F (HSV-1) and cytopathic bovine viral diarrhea virus type-1 NADL strain (BVDV, ATCC VR534) were propagated in Vero and MDBK cells, respectively.

4.2.2. Cytotoxicity assays

Vero, MDBK and HepG2 2.215 cells (1×10^4, 1×10^4 and 4×10^4 and cells per well, respectively) were seeded in 96-well plates, 24 h later increasing concentrations of the compounds were added and they were incubated at 37 °C with 5% CO_2. After 2-days incubation for Vero cells, or 3-days incubation for MDBK cells or 9-days incubation for HepG2 2.2.15 cells, the cell viability was measured by CellTiter 96 AQueous (Promega, Madison, Wis.). The

cytotoxic concentration 50% (CC_{50}) was calculated for compounds **4**, **6**, **7**, **9**, **11**, **12**, **15**–**20**.

4.2.3. Antiviral assays

The assayed compounds were **4**, **6**, **7**, **9**, **11**, **12**, **15–20**. The antiviral activity against HSV-1 and BVDV was evaluated by viral plaque or cytopathic effect (CPE) reduction assays, respectively.

Briefly, a 90% confluent monolayer of Vero cells in 24 well plates was infected with 100 PFU of HSV-1/well. After 1 h of adsorption period, the viral inoculum was removed and overlay medium with 0.5% (w/v) methylcellulose, with or without serial concentrations of each compound, was added (100–3.125 μM). Acyclovir was used as positive control. After 2 days of incubation at 37 °C and 5% CO_2, the cell monolayers were fixed and stained. The number of viral plaques was counted.

For BVDV, the antiviral assay was performed as described previously.[25] Briefly, subconfluent MDBK cells in 96 wells plates were infected with BVDV NADL cp (m.o.i = 0.01), in presence or absence of infection medium containing serial concentrations of carbanucleoside analogues (100–3.125 μM). Ribavirin was used as positive control. Uninfected and infected cells without compound (virus control) were included in each plate. After 3 days of incubation at 37 °C and 5% CO_2, the cellular viability was determined by MTS/PMS method. The % of I of viral cytopathic effect (CPE) at 100 μM of each compound was calculated.

For HBV, the antiviral assay was performed as described previously.[26] Briefly, HepG2 2.2.15 cells on 96-well cell culture plates were treated with two fold serial dilution of each compound (100–3.125 μM) or with medium. After 9 days of incubation the extracellular media were collected, clarified (3000×*g*, 10 min) and the supernatants were stored at −20 °C to further analysis. Lamivudine was used as positive control.

4.2.4. ELISA assay of HBsAg

Viral HBV surface antigen (HBsAg) levels in culture medium were measured using enzyme-linked immunosorbent assay (ELISA) ETIMAK-4, (Diasorin, Italy) according to the manufacture's instructions. The absorbance was measured at 450/630 nm using a microplate reader (Biotek Synergy HT).

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