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Short communication

Synthesis and cytostatic activity of purine nucleosides derivatives of allofuranose

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ABSTRACT

Several new purine nucleosides derivatives of allofuranose were prepared according to Vorbrüggen method, starting from 1,2,5,6-di-*O*-isopropylidene-α-D-allofuranose and using 1,2,3,5,6-pentaacetoxy-β-D-allofuranose as key intermediate. The synthesized allofuranosyl nucleosides, as well as some acetyl derivatives, were evaluated for their cytotoxicity in vitro in three human cancer cell lines (MCF-7, Hela-229 and HL-60). Among the studied compounds the 9-(2,3,5,6-tetra-*O*-acetyl-β-D-allofuranosyl)-2,6-dichloropurine (**9**) was the most potent one on the three cell lines evaluated, being its activity against HL-60 cells similar to cisplatin.

1. Introduction

Purine nucleoside analogues play an important role as cytostatic agents. Thus, cladribine (**1a**) and fludarabine (**1b**) are used in oncology for the treatment of both hematological malignant diseases and some solid tumors [1] (Fig. 1). In the last years a number of new purine nucleosides have been developed. Some of these compounds, such as nelarabine (**1c**) or clofarabine (**1d**), are currently under investigation in clinical studies [2]. For instance, clofarabine is a new deoxyadenosine analogue that has demonstrated single-agent anticancer activity in pediatric and also in adult acute leukaemias [3].

Purine nucleoside analogues, in general, act as antimetabolites after their metabolic phosphorylation to the corresponding 5′-triphosphates, compete with physiological nucleosides and interact with a number of cellular targets to induce cytotoxicity. All purine nucleoside analogues develop a similar mechanism of cytotoxicity both in proliferating and quiescent cells, such as inhibition of DNA synthesis and repair, and accumulation of DNA strand breaks, being apoptosis the end-point of their action [4,5]. However, significant differences exist not only in the target enzymes but also in the drug–target interactions of these antimetabolites, which could explain their different biological activities [4].

Most of purine derivatives described before show an amino group or similar (alkoxy, chloro) at 6th position of the heterocyclic base which could be essential to establish hydrogen bonds with polymerases and other key enzymes of nucleic acid metabolism [6]. However, purine nucleosides bearing C-substituents at C-6 (aryl, alkyl, benzyl, or heteroaryl) also possess significant cytostatic effects [6,7]. In addition, substitution at C-2 of the purine ring by halogen seems to be favorable [8]. The sugar moieties have been also modified for the development of new purine nucleosides, in particular 2′ and 3′ positions. A number of 2′ or 3′ modified nucleosides have shown potent cytostatic activity, such as 3′-*C*-methyl derivatives [9], 2′-*O*-alkenyl analogues [10] as well as 2′-deoxy derivatives and nucleosides with arabino configuration, that in many cases have proven to be inhibitors of multienzymes [11]. On the other hand, structural changes at 4′ position of the sugar moiety have been studied with minor profusion although several 4′-modified nucleoside analogues have been synthesized and some of them have shown significant biological properties [12–14].

Regarding this aspect, it is necessary to emphasize the potential interest of hexofuranosyl nucleosides because of their great structural similitude with the ribosyl nucleosides. The main difference between both types of analogues is the presence at 4′ of an ethylene glycol group replacing the habitual hydroxymethyl group [15]. Some hexofuranosyl nucleosides have been previously synthesized and biologically evaluated as antiviral agents [16], however there are not recent studies about the potential cytostatic activity of this class of derivatives [17]. A reason for the lack of cytotoxicity

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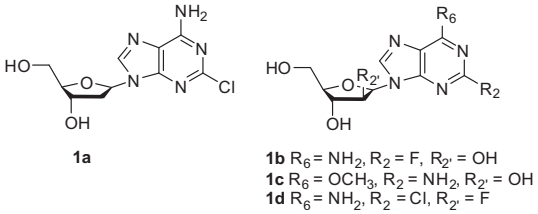


Fig. 1. Nucleoside cytostatic drugs.

data could be the fact that they are currently considered as conformationally restricted acyclic nucleosides [18], and structurally related to an important group of antiviral agents.

Taking into account that nucleoside analogues are among the most complex and promising cytostatic agents and as part of our research program in this field [19], we are describing in this work the synthesis and cytostatic activity of several purine nucleosides derivatives of allofuranose of structure **2** (Fig. 2). The selection of substituents at the purine nucleus was based on the analogy to some of the most active compounds known.

2. Results and discussion

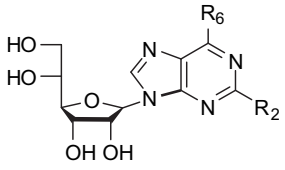
2.1. Chemistry

The allofuranosyl nucleosides **2a**–**h** were prepared according to Vorbrüggen method [20] using 1,2,3,5,6-pentaacetoxy-β-D-allofuranose (**6β**) as key intermediate.

The allofuranose derivative **6β** was obtained in good yield from the commercially available 1,2,5,6-di-*O*-isopropylidene-α-D-allofuranose (**3**) in four steps that included a sequence of two selective *O*-isopropylidene deprotections [21] each one followed of corresponding acetylation (Scheme 1). Thus, compound **6** was obtained as a mixture of α- and β-anomers (α:β ratio 1:3.5), which were separated by column chromatography [22].

The acetyl protected nucleoside **7** was obtained in 41% yield by coupling between furanose **6β** and the adequate purine, following Vorbrüggen conditions, as shown in Scheme 2. Thus, 6-chloropurine was silylated *in situ* using the conventional methodology [23] and then was condensed with **6β**. The reaction was performed under reflux in acetonitrile with the presence of trimethylsilyl triflate (TMSOTf). Two critical factors for the success of this reaction were the solvent and Lewis acid, since the use of dichloromethane under similar reaction conditions resulted in a complicated mixture of products from which the nucleoside **7** was isolated in only a 17% yield. Moreover, when $SnCl_2$ was used instead of TMSOTf compound **7** was not obtained.

Similarly, The acetyl protected nucleoside **9** was obtained in a 74% yield when furanose **6β** was treated with silylated 2,6-dichloropurine. Deacetylation of **7** was performed with methanolic ammonia at 0 °C to provide compound **2a** in 73% yield. However, ammonia was not suitable for selective deacetylation of **9** because of the high reactivity of the chlorine atom at C-6 with



2a $R_6 = Cl, R_2 = H$
2b $R_6 = Cl, R_2 = Cl$
2c $R_6 = NH_2, R_2 = H$
2d $R_6 = NH_2, R_2 = Cl$
2e $R_6 = NHCH_3, R_2 = H$
2f $R_6 = NHCH_3, R_2 = Cl$
2g $R_6 = Ph, R_2 = H$
2h $R_6 = OEt, R_2 = Cl$

Fig. 2. Purine nucleosides derivatives of allofuranose synthesized.

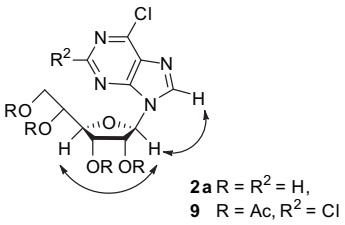


Fig. 3. Proton–proton interactions observed in the NOE spectrums of compounds **2a** and **9**.

nucleophilic reagents. Thus, treatment of **9** with NH_3 0.5 M in dioxane at r.t. provided the aminopurine derivative peracetylated **11**. On the other hand, chemoselective removal of O-acetyl groups in compound **9** was achieved using dibutyltin oxide (DBTO) [24]. When **9** was refluxed for 48 h with an excess of DBTO in toluene the chloropurine analogue **2b** was obtained as mainly product (49%) together with the 2′,3′-diacetyl derivative **10** (32%). Both compounds can be easily separated by flash chromatography.

In addition, treatment of nucleosides **7** and **9** with 25% aqueous ammonia at reflux resulted in substitution of the chloro at C-6 together with deacetylation providing the aminopurine analogues **2c** [15] and **2d**. Similarly, reaction of **7** and **9** with an excess of methylamine hydrochloride and triethylamine in MeOH gave the nucleosides **2e** and **2f** in 78% and 62% yields respectively.

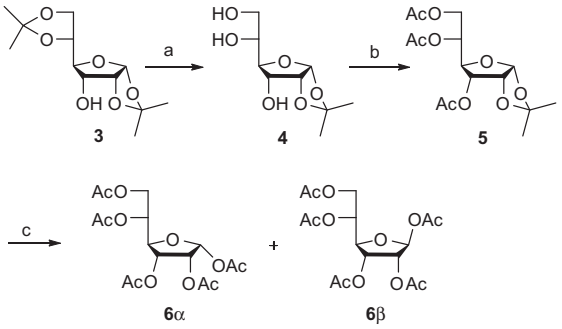
The 6-phenyl nucleoside **2g** was prepared in good yield in two steps, a Suzuki–Miyaura type coupling of **7** with phenylboronic acid using anhydrous K_2CO_3 and $Pd(Ph_3P)_4$ in toluene [6] to give the 2′,3′,5′,6′-tetra-*O*-acetyl-6-phenyl nucleoside **8** in 62% yield, followed by deacetylation with methanolic ammonia at room temperature.

Finally, the treatment of **9** at room temperature with K_2CO_3 in a mixture of dichloromethane-ethanol gave the 2-chloro-6-ethoxy derivative **2h**.

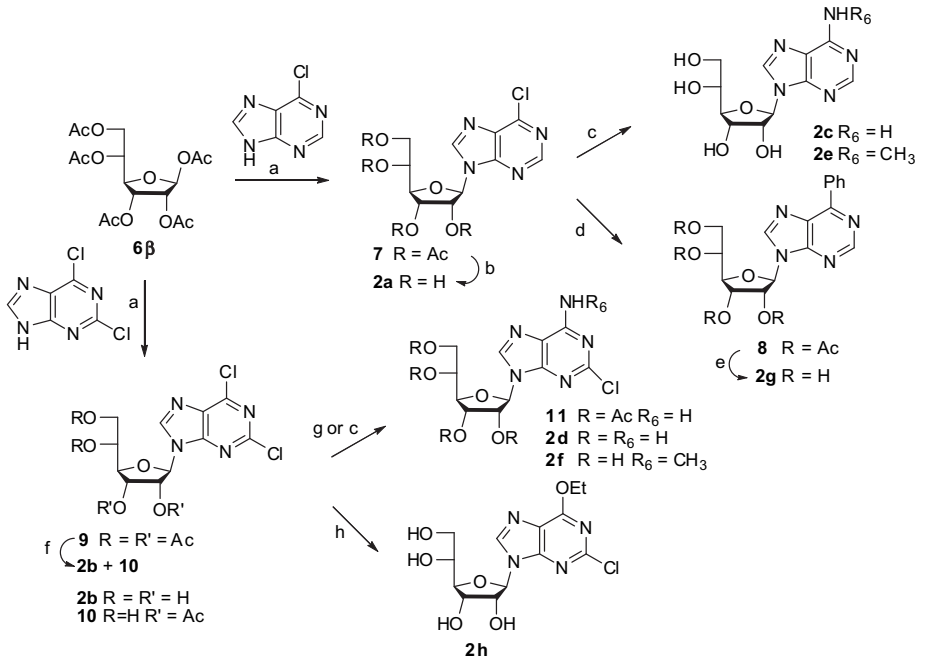
The stereochemistry of two series of derivatives was further established by NOE correlation experiments. Thus, in the NOE spectrum of **2a**, as well as in the NOE spectrum of **9**, strong NOE effects were observed between the anomeric proton H-1′ and H-4′, which indicate that both nucleosides are β-isomers (Fig. 3). Moreover, the NOE effects observed for both compounds between H-8 and H-1′ provide evidence about the N-9 substitution of the purine ring [25].

2.2. Cytostatic activity

The synthesized allofuranosyl nucleosides **2a**–**h**, as well as the acetyl derivatives **7**–**11**, were evaluated for their cytotoxicity *in vitro* in three human cancer cell lines: breast cancer (MCF-7), cervix



Scheme 1. Reagents and conditions: (a) 80% aq. AcOH, r.t., 92%; (b) Ac_2O, Py, r.t., 96%; (c) (i) 80% aq. TFA, 0 °C, (ii) Ac_2O, Py, DMAP, r.t., 84%.



Scheme 2. Reagents and conditions: (a) HMDS, $(NH_4)_2SO_4$, TMSOTf, CH_3CN, reflux, 41% (**7**), 74% (**9**); (b) NH_3 2 M MeOH, 0 °C, 73%; (c) NH_4OH 25%, CH_3CN, reflux, 65% (**2c**), 60% (**2d**); $CH_3NH_2 \cdot HCl$, Et_3N, MeOH, 78% (**2e**), 62% (**2f**); (d) $PhB(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, toluene, 100 °C, 62%; (e) NH_3 2 M MeOH, r.t., 90%; (f) DBTO, toluene, 100 °C, 49% (**2b**), 32% (**10**); (g) NH_3 0.5 M, dioxane, r.t., 70% (**11**); (h) K_2CO_3, CH_2Cl_2-EtOH, r.t., 50%.

cancer (Hela-229) and promyelocytic leukaemia (HL-60) using cisplatin as the reference compound and the results are included in Table 1.

Among all the acetyl-free nucleosides tested (compounds **2a**–**h**) only the 2,6-dichloropurine derivative **2b** showed moderate cytostatic activity against MCF-7 and Hela-229 cells with similar IC_{50} values (54.2 and 47.1 μM respectively), being inactive against HL-60 cell line at concentrations below of 100 μM. However, their acetyl derivatives (compounds **9**–**10**) showed good activity against the three tested cell lines with IC_{50} values ranging from 16.1 to 45.0 μM. On the other hand, the acetylated nucleoside **7** that contains a chlorine atom at C-6 displayed significant cytostatic activity that was selective against HL-60 cells (IC_{50} 79.3 μM).

In conclusion, a new series of purine nucleosides derivatives of allofuranose were prepared in good yield according to Vorbrüggen method and using 1,2,3,5,6-pentaacetoxy-β-D-allofuranose (**6β**) as key intermediate. Cytotoxicity studies revealed the 2,6-dichloropurine derivative **2b** as the most interesting of the target compounds, these results are in accordance with previous studies for the related compounds [8,26]. Compound **2b** showed lower activity than their acetyl derivatives **9** and **10**. Thus, the tetra-*O*-acetyl derivative **9** was the most potent one on the three cell lines evaluated, being its activity against HL-60 cells similar to cisplatin. The cytostatic activity of these chloropurine derivatives appears to be modulated by inclusion of acetyl groups in the sugar moiety. Acetyl groups habitually increase the lipophilicity of drugs supporting their cell penetration. This could also explain the activity shown by the acetyl protected nucleoside **7**.

Table 1
Cytostatic activity for the nucleosides **2a-h** and for the acetyl derivatives **7-11**.

Compound	IC_{50} (μM)[a]		
	MCF-7	Hela-229	HL-60
2a	> 100	> 100	> 100
2b	54.2 (± 3.7)	47.1 (± 2.2)	> 100
2c	> 100	> 100	> 100
2d	> 100	> 100	> 100
2e	> 100	> 100	> 100
2f	> 100	> 100	> 100
2g	> 100	> 100	> 100
2h	> 100	> 100	> 100
7	> 100	> 100	79.3 (± 5.1)
8	> 100	> 100	> 100
9	22.4 (± 0.8)	24.2 (± 1.1)	16.1 (± 0.9)
10	36.3 (± 1.0)	36.3 (± 1.2)	45.0 (± 0.7)
11	> 100	> 100	> 100
Cisplatin	9.2 (± 0.6)	0.9 (± 0.2)	14.8 (± 1.2)

[a] Values are means of three experiments, standard deviation is given in parentheses.

3. Experimental section

3.1. Chemistry

All starting materials and common laboratory chemicals were purchased from commercial sources and used without further purification. All solvents were distilled and dried according to standard procedures. ^1H and ^{13}C NMR spectra were recorded on a Bruker ARX400 and Bruker Avance DPX400 instruments, using TMS as internal standard [chemical shifts (δ) in ppm, J in Hz]. Assignment of the signals was performed by NOE, COSY, DEPT, HMQC experiments. High resolution mass spectra were recorded using a Bruker microTOF focus spectrometer. Silica gel (Merck 60, 230–400 mesh) was used for flash chromatography (FC). Preparative thin layer chromatography was performed on Analtech silica gel GF 1000 μm plates. Analytical TLC was performed on plates precoated with silica gel (Merck 60 F254, 0.25 mm).

3.2. Preparation of 9-(2,3,5,6-tetra-O-acetyl-β-D-allofuranosyl)-6-chloropurine (7)

A mixture of 6-chloropurine (198 mg, 1.28 mmol), HMDS (2 mL) and $(NH_4)_2SO_4$ (5 mg) was refluxed for 2 h and then the solvent

was removed in vacuo. To a mixture of the silylated 6-chloropurine in CH_3CN (4 mL) a solution of compound **6β** (191 mg, 0.49 mmol) in CH_3CN (3 mL) and TMSOTf (0.13 mL, 0.73 mmol) was added. The reaction mixture was stirred at room temperature for 1 h and then refluxed for 15 h, followed by quenching with saturated aq. $NaHCO_3$ (10 mL). The product was extracted with EtOAc (3 × 10 mL). The organic layer was dried over Na_2SO_4, and concentrated to dryness. The residue was purified by column chromatography on silica gel (hexane–ethyl acetate, 1:1) to afford **7** (96 mg, 41%) as a colorless oil. R_f = 0.15 (hexane–ethyl acetate, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.76 (s, 1H, H-purine), 8.18 (s, 1H, H-purine), 6.13 (d, *J* = 6.4 Hz, 1H, H-1′), 6.12–6.07 (m, 1H, H-2′), 5.80 (dd, *J* = 5.3, 3.6 Hz, 1H, H-3′), 5.49–5.45 (m, 1H, H-5′), 4.44 (dd, *J* = 12.2, 4.0 Hz, 1H, 1H-6′), 4.39 (dd, *J* = 5.3, 3.6 Hz, 1H, H-4′), 4.10 (dd, *J* = 12.2, 5.2 Hz, 1H, 1H-6′), 2.17 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.03 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 169.8, 169.3, 169.1, 152.3, 151.8, 151.2, 144.0, 132.5, 86.6, 81.5, 71.9, 70.5, 70.0, 61.8, 20.9, 20.6, 20.5, 20.3; HRMS-ESI: *m/z* $[M + H]^+$ calcd for $C_{19}H_{22}ClN_4O_9$: 485.10698, found: 485.10883.

3.3. Preparation of 9-(2,3,5,6-tetra-O-acetyl-β-D-allofuranosyl)-2,6-dichloropurine (**9**)

Obtained from 2,6-dichloropurine (271 mg, 1.39 mmol), **6β** (211 mg, 0.54 mmol) and TMSOTf (0.15 mL, 0.81 mmol) following the same procedure as for preparation of compound **7**. The residue was purified by column chromatography on silica gel (hexane–ethyl acetate, 1.5:1) to afford **9** (208 mg, 74%) as a colorless oil. R_f = 0.24 (hexane–ethyl acetate, 1:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.20 (s, 1H, H-8), 6.13 (d, *J* = 6.5 Hz, 1H, H-1′), 5.88–5.85 (m, 1H, H-2′), 5.73 (dd, *J* = 5.6, 3.3 Hz, 1H, H-3′), 5.47–5.44 (m, 1H, H-5′), 4.46 (dd, *J* = 12.3, 3.8 Hz, 1H, 1H-6′), 4.38 (dd, *J* = 5.6, 3.3 Hz, 1H, H-4′), 4.12 (dd, *J* = 12.3, 5.2 Hz, 1H, 1H-6′), 2.17 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.04 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.4, 169.8, 169.3, 169.2, 153.5, 152.6, 152.5, 144.0, 131.5, 86.2, 81.6, 72.3, 70.4, 69.9, 61.8, 20.8, 20.6, 20.5, 20.3; HRMS-ESI: *m/z* $[M + H]^+$ calcd for $C_{19}H_{21}Cl_2N_4O_9$: 519.06801, found: 519.07004.

3.4. Preparation of 9-β-D-allofuranosyl-6-chloropurine (**2a**)

To a solution of compound **7** (15 mg, 0.03 mmol) in MeOH (0.5 mL) was added NH_3 2 M in MeOH (3 mL) and the reaction mixture was stirred at 0 °C for 4 h. After removal of the solvent, the residue was purified by preparative thin layer chromatography on silica gel (CH_2Cl_2-MeOH, 9:1) to give **2a** (7 mg, 73%) as a white solid. R_f = 0.15 (CH_2Cl_2-MeOH, 9:1). ^{1}H NMR (DMSO-d_6): δ = 8.94 (s, 1H, H-purine), 8.81 (s, 1H, H-purine), 6.02 (d, *J* = 6.5 Hz, 1H, H-1′), 5.50 (d, *J* = 6.1 Hz, 1H, OH-2′), 5.25 (d, *J* = 4.9 Hz, 1H, OH-5′), 5.21 (d, *J* = 4.6 Hz, 1H, OH-3′), 4.63–4.60 (m, 2H, H-2′, OH-6′), 4.28–4.25 (m, 1H, H-3′), 4.03–4.01 (m, 1H, H-4′), 3.80–3.70 (m, 1H, H-5′), 3.40–3.36 (m, 2H, H-6′). ^{13}C NMR (DMSO-d_6): δ = 151.7 (2C), 149.3, 145.8, 131.3, 87.4, 86.1, 74.0, 71.4, 69.4, 62.4. HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{11}H_{14}ClN_4O_5$: 317.06472; found: 317.06466.

3.5. Preparation of 9-(2,3-di-O-acetyl-β-D-allofuranosyl)-2,6-dichloropurine (**10**) and 9-β-D-allofuranosyl-2,6-dichloropurine (**2b**)

A mixture of **9** (30 mg, 0.06 mmol) and Bu_2SnO (124 mg, 0.49 mmol) in toluene (8 mL) was refluxed for 48 h and then the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2-MeOH, 95:5) to afford **10** (8 mg, 32%) and **2b** (10 mg, 49%) both as a colorless oils. Compound **10**: R_f = 0.68 (CH_2Cl_2-MeOH, 9:1);^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.94 (s, 1H, H-8), 6.24 (d, *J* = 6.8 Hz, 1H, H-1′), 5.85–5.83 (m, 1H, H-2′), 5.65 (dd, *J* = 5.4, 2.1 Hz, 1H, H-3′), 5.56 (d, *J* = 5.1 Hz, 1H, OH-5′), 4.78 (t, *J* = 5.5 Hz, 1H, OH-6′), 4.32–4.30 (m, 1H, H-4′), 3.82–3.86 (m, 1H, H-5′), 3.43–3.39 (m, 2H, H-6′), 2.14 (s, 3H, CH_3), 1.97 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.4, 169.1, 153.1, 151.3, 150.1, 146.1, 131.0, 85.1, 84.3, 73.2, 71.1, 70.2, 62.1; HRMS-ESI: *m/z* $[M + H]^+$ calcd for $C_{15}H_{17}Cl_2N_4O_7$: 435.04688, found: 435.04665. Compound **2b**: R_f = 0.28 (CH_2Cl_2-MeOH, 9:1); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.97 (s, 1H, H-8), 5.95 (d, *J* = 6.2 Hz, 1H, H-1′), 5.55 (d, *J* = 6.0 Hz, 1H, OH-2′), 5.25–5.23 (m, 2H, OH-3′, OH-5′), 4.65 (t, 1H, *J* = 5.6 Hz, OH-6′), 4.56–4.52 (m, 1H, H-2′), 4.26–4.22 (m, 1H, H-3′), 4.02–3.99 (m, 1H, H-4′), 3.77–3.73 (m, 1H, H-5′), 3.44–3.40 (m, 2H, H-6′); ^{13}C NMR (100 MHz, DMSO-d_6): δ = 153.2, 151.2, 149.9, 146.4, 130.9, 87.4, 86.0, 74.2, 71.3, 69.3, 62.4; HRMS-ESI: *m/z* $[M + Na]^+$ calcd for $C_{11}H_{12}Cl_2N_4NaO_5$: 373.16350, found: 373.16383.

3.6. Preparation of 9-β-D-allofuranosyladenine (**2c**)

To a solution of compound **7** (38 mg, 0.08 mmol) in CH_3CN (1 mL) was added 25% NH_4OH (6 mL). The reaction mixture was stirred at 100 °C in a sealed tube for 13 h. After removal of the solvent, the residue was purified by preparative thin layer chromatography on silica gel (CH_2Cl_2-MeOH, 8:2) to give **2c** (15 mg, 65%) as a white solid. R_f = 0.30 (CH_2Cl_2-MeOH, 8:2). ^{1}H NMR (DMSO-d_6): δ = 8.22 (s, 1H, H-purine), 8.01 (s, 1H, H-purine), 7.25 (s, 2H, NH_2), 5.73 (d, *J* = 7.3 Hz, 1H, H-1′), 5.73–5.70 (m, 1H, OH-5′), 5.27 (d, *J* = 6.6 Hz, 1H, OH-2′), 5.04 (d, *J* = 3.8 Hz, 1H, OH-3′), 4.57–4.51 (m, 2H, H-2′, OH-6′), 4.08–4.06 (m, 1H, H-3′), 3.92–3.90 (m, 1H, H-4′), 3.60–3.58 (m, 1H, H-5′), 3.31–3.28 (m, 2H, H-6′). ^{13}C NMR (DMSO-d_6): δ = 156.6, 152.7, 149.4, 140.6, 119.9, 87.9, 87.1, 73.7, 72.4, 69.9, 62.8. HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{11}H_{16}N_5O_5$: 298.11460; found: 298.11598.

3.7. Preparation of 9-β-D-allofuranosyl-N^6-methyladenine (**2e**)

To a solution of compound **7** (20 mg, 0.04 mmol) in MeOH (1 mL) was added methylamine hydrochloride (139 mg, 2.06 mmol) and Et_3N (0.43 mL). The reaction mixture was stirred at 65 °C in a sealed tube for 24 h. After removal of the solvent, the residue was purified by preparative thin layer chromatography on silica gel (CH_2Cl_2-MeOH, 8:2) to give **2e** (10 mg, 78%) as a white solid. R_f = 0.31 (CH_2Cl_2-MeOH, 8:2). ^{1}H NMR (DMSO-d_6): δ = 8.32 (s, 1H, H-purine), 8.22 (s, 1H, H-purine), 7.85 (s, 1H, NH), 5.84 (d, *J* = 7.4 Hz, 1H, H-1′), 5.84–5.82 (m, 1H, OH-5′), 5.36 (d, *J* = 6.7 Hz, 1H, OH-2′), 5.12 (d, *J* = 3.8 Hz, 1H, OH-3′), 4.67–4.61 (m, 2H, OH-6′, H-2′), 4.20–4.16 (m, 1H, H-3′), 4.03–4.01 (m, 1H, H-4′), 3.74–3.70 (m, 1H, H-5′), 3.43–3.41 (m, 2H, H-6′), 2.96 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ = 155.1, 152.2, 147.9, 139.9, 120.0, 87.5, 86.6, 73.3, 71.9, 69.5, 62.3, 26.9. HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{12}H_{18}N_5O_5$: 312.13025; found: 312.13079.

3.8. Preparation of 9-β-D-allofuranosyl-2-chloroadenine (**2d**)

Obtained from **9** (27 mg, 0.05 mmol) following the same procedure as for preparation of compound **2c** from **7**. The residue was purified by preparative thin layer chromatography on silica gel (CH_2Cl_2-MeOH, 8:2) to afford **2d** (10 mg, 60%) as a white solid. R_f = 0.36 (CH_2Cl_2-MeOH, 8:2). ^{1}H NMR (DMSO-d_6): δ = 8.37 (s, 1H, H-8), 7.85 (s, 2H, NH_2), 5.79 (d, *J* = 7.2 Hz, 1H, H-1′), 5.41–5.37 (m, 1H, OH-2′), 5.23–5.21 (m, 1H, OH-5′), 5.16–5.14 (m, 1H, OH-3′), 4.61–4.59 (m, 1H, OH-6′), 4.55–4.53 (m, 1H, H-2′), 4.20–4.18 (m, 1H, H-3′), 3.99–3.97 (m, 1H, H-4′), 3.72–3.71 (m, 1H, H-5′), 3.42–3.40 (m, 2H, H-6′). ^{13}C NMR (DMSO-d_6): δ = 156.7, 152.9, 150.3, 140.1, 118.1, 86.6, 86.1, 73.6, 71.6, 69.5, 62.3. HRMS (ESI) *m/z* $[M + H]^+$ calcd for $C_{11}H_{15}ClN_5O_5$: 332.07562; found: 332.07477.

3.9. Preparation of 9-β-D-allofuranosyl-2-chloro-N^6-methyladenine (**2f**)

Obtained from **9** (22 mg, 0.04 mmol) following the same procedure as for preparation of compound **2e** from **7**. The residue was purified by column chromatography on silica gel (CH_2Cl_2-MeOH, 93:7) to afford **2f** (9 mg, 62%) as a white solid. R_f = 0.38 (CH_2Cl_2-MeOH, 8:2). ^{1}H NMR (DMSO-d_6): δ = 8.37 (s, 1H, H-8), 8.31 (s, 1H, NH), 5.79 (d, J = 7.0 Hz, 1H, H-1′), 5.40 (d, J = 6.6 Hz, 1H, OH-2′), 5.23 (d, J = 4.7 Hz, 1H, OH-5′), 5.15 (d, J = 4.5 Hz, 1H, OH-3′), 4.60 (t, J = 5.6 Hz, 1H, OH-6′), 4.56–4.52 (m, 1H, H-2′), 4.21–4.17 (m, 1H, H-3′), 3.99–3.97 (m, 1H, H-4′), 3.72–3.68 (m, 1H, H-5′), 3.44–3.40 (m, 2H, H-6′), 2.93 (d, J = 4.4 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ = 155.5, 153.2, 149.2, 139.9, 118.7, 86.7, 86.1, 73.6, 71.6, 69.5, 62.4, 27.1. HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{12}H_{17}ClN_5O_5$: 346.09127; found: 346.09224.

3.10. Preparation of 9-β-D-allofuranosyl-6-phenylpurine (**2g**)

To a solution of compound **8** (15 mg, 0.03 mmol) in MeOH (0.5 mL) was added NH_3 2 M in MeOH (2 mL) and the reaction mixture was stirred at room temperature for 4 h. After removal of the solvent, the residue was purified by column chromatography on silica gel (CH_2Cl_2-MeOH, 9:1) to give **2g** (9 mg, 90%) as a white solid. R_f = 0.34 (CH_2Cl_2-MeOH, 9:1). ^{1}H NMR (DMSO-d_6): δ = 9.01 (s, 1H, H-purine), 8.92 (s, 1H, H-purine), 8.83–8.81 (m, 2H, H-phenyl), 7.63–7.60 (m, 3H, H-phenyl), 6.07 (d, J = 6.7 Hz, 1H, H-1′), 5.52 (d, J = 6.3 Hz, 1H, OH-2′), 5.35 (d, J = 4.8 Hz, 1H, OH-5′), 5.23 (d, J = 4.5 Hz, 1H, OH-3′), 4.71–4.69 (m, 1H, H-2′), 4.65 (t, J = 5.6 Hz, 1H, OH-6′), 4.28–4.26 (m, 1H, H-3′), 4.04–4.02 (m, 1H, H-4′), 3.79–3.75 (m, 1H, H-5′), 3.45–3.41 (m, 2H, H-6′). ^{13}C NMR (DMSO-d_6): δ = 153.1, 152.3, 151.9, 145.1, 135.2, 131.2, 130.9, 129.4, 128.7, 87.0, 86.1, 73.8, 71.6, 69.5, 62.5. HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{17}H_{19}N_4O_5$: 359.13500; found: 359.13476.

3.11. Preparation of 9-β-D-allofuranosyl-2-chloro-6-ethoxypurine (**2h**)

A mixture of compound **9** (15 mg, 0.03 mmol), K_2CO_3 (7 mg, 0.05 mmol), CH_2Cl_2 (1 mL) and EtOH (3 mL) was stirred at room temperature for 4 h. After removal of the solvent, the residue was purified by preparative thin layer chromatography on silica gel (CH_2Cl_2-MeOH, 98:2) to give **2h** (5 mg, 50%) as a white solid. R_f = 0.26 (CH_2Cl_2-MeOH, 9:1). ^{1}H NMR (DMSO-d_6): δ = 8.19 (s, 1H, H-8), 5.84 (d, J = 5.9 Hz, 1H, H-1′), 4.62 (q, J = 7.0 Hz, 2H, CH_2), 4.60–4.56 (m, 1H, H-2′), 4.41–4.39 (m, 1H, H-3′), 4.20–4.18 (m, 1H, H-4′), 4.02–3.98 (m, 1H, H-5′), 3.48–3.44 (m, 2H, H-6′), 1.46 (t, J = 7.0 Hz, 3H, CH_3). HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{13}H_{18}ClN_4O_6$: 361.09094; found: 361.09161.

3.12. Preparation of 9-(2,3,5,6-tetra-O-acetyl-β-D-allofuranosyl)-6-phenylpurine (**8**)

To a solution of compound **7** (20 mg, 0.04 mmol) in toluene (4 mL) was added K_2CO_3 (17 mg, 0.12 mmol), $PhB(OH)_2$ (15 mg, 0.12 mmol) and $Pd(PPh_3)_4$ (9 mg, 0.008 mmol). The reaction mixture was stirred at 100 °C for 48 h. After removal of the solvent, the residue was purified by column chromatography on silica gel (hexane–ethyl acetate, 1:1) to give **8** (13 mg, 62%) as a white solid. R_f = 0.35 (hexane–ethyl acetate, 1:1). ^{1}H NMR ($CDCl_3$): δ = 9.03 (s, 1H, H-purine), 8.76–8.74 (m, 2H, H-phenyl), 8.18 (s, 1H, H-purine), 7.57–7.53 (m, 3H, H-phenyl), 6.20–6.16 (m, 2H, H-1′, H-2′), 5.87–5.85 (m, 1H, H-3′), 5.52–5.50 (m, 1H, H-5′), 4.48 (dd, J = 12.2, 4.0 Hz, 1H, 1H-6′), 4.42–4.40 (m, 1H, H-4′), 4.08 (dd, J = 12.2, 5.3 Hz, 1H, 1H-6′), 2.18 (s, 3H, CH_3), 2.15 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.05 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$): δ = 170.4, 169.8, 169.4, 169.2, 155.6, 152.7, 152.0, 143.0, 135.2, 131.8, 131.2, 129.8, 128.7, 86.2, 81.3, 71.9, 70.6, 70.2, 61.9, 20.9, 20.7, 20.6, 20.3. HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{25}H_{27}N_4O_9$: 527.17725; found: 527.17671.

3.13. Preparation of 9-(2,3,5,6-tetra-O-acetyl-β-D-allofuranosyl)-2-chloroadenine (**11**)

A solution of compound **9** (10 mg, 0.02 mmol) in NH_3 0.5 M in dioxane (3 mL) was stirred at room temperature for 48 h. After removal of the solvent, the residue was purified by preparative thin layer chromatography on silica gel (CH_2Cl_2-MeOH, 96:4) to give **11** (7 mg, 70%) as a white solid. R_f = 0.65 (CH_2Cl_2-MeOH, 9:1). ^{1}H NMR (DMSO-d_6): δ = 8.39 (s, 1H, H-8), 7.93 (s, 2H, NH_2), 6.15 (d, J = 6.1 Hz, 1H, H-1′), 5.94–5.90 (m, 1H, H-2′), 5.68–5.64 (m, 1H, H-3′), 5.44–5.40 (m, 1H, H-5′), 4.34–4.29 (m, 2H, H-4′, 1H-6′), 4.08 (dd, J = 12.3, 5.6 Hz, 1H, 1H-6′), 2.13 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 1.99 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ = 169.9, 169.5, 169.2, 169.1, 156.8, 153.2, 150.0, 140.6, 118.3, 85.1, 79.8, 71.3, 69.8, 69.4, 61.4, 20.5, 20.4, 20.3, 20.1. HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{19}H_{23}ClN_5O_9$: 500.11788; found: 500.11888.

3.14. Cytotoxicity assays in vitro

Cytotoxicity effects on MCF-7, Hela-229 and HL-60 cells were determined by means of a colorimetric microculture assay, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay [27–29]. Briefly, cells were seeded in 96-well plates (10^4 cells per well for MCF-7 and HL-60 cell lines, and 4×10^3 for Hela-229 cell line), incubated for 24 h in the culture medium and treated at 37 °C for 96 h (MCF-7), 24 h (HL-60) or 48 h (Hela-229) with varying doses of evaluated compounds and the reference drug, cisplatin, dissolved in DMSO. Three wells were used for each of the variants tested. Aliquots of MTT solution in phosphate-buffered saline (10 μL) were added to each well and incubated for 4 h. The color formed was quantified by a spectrophotometric plate reader (Tecan Ultra evolution) at 595 nm wavelength. In all experiments, DMSO controls were included. The percentage cell viability was calculated by dividing the average absorbance of cells treated with a compound by that of the control. 50% Inhibitory concentrations (IC_{50}) were calculated from concentration-effect curves by using GraphPad Prism software, version 2.01. Correlation coefficients (r^2) were higher than 0.998 for all compounds tested.

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