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Original article

Synthesis, antiviral activity, and stability of nucleoside analogs containing tricyclic bases

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ABSTRACT

A series of 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*A*]purine nucleosides (tricylic nucleosides) were synthesized from 9-[4-α-(hydroxymethyl)cyclopent-2-ene-1-α-yl]guanine (CBV) **5**, (−)-β-D-(2*R*,4*R*)-1,3-dioxolane-guanosine (DXG) **6**, 3′-azido-3′-deoxy-guanosine (AZG) **7**, and 2′-*C*-methylguanosine **8**. Their *in vitro* activity against HIV and HCV was evaluated and correlated to their ability to degrade to their purine counterpart.

1. Introduction

The alkylation–condensation of guanosine nucleosides **1** with phenylacyl bromide is known to produce the corresponding 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*A*]purine nucleoside **2** (or simply called tricyclic nucleoside). This reaction has been extensively studied using various substrates such as 2′-deoxyguanosine **1a** [1] and guanosine **1b**, the cyclopropyl-guanosine **1c** [2], 9-(2-phosphonyl–methoxyethyl)guanine (PMEG) **1d** [3] and also the FDA approved ganciclovir (GCV) **1e** and acyclovir (ACV) **1f** [4–13] (Scheme 1). The corresponding highly conjugated compounds **2** show some fluorescent properties and, surprisingly in some cases, exhibited potent and selective antiviral activity. Over the years, various studies have established the antiviral influence of the substitution on the tricylic moiety. The data obtained from more than 70 ACV and GCV analogs, indicated that compounds **3** and **4** were as active as their parent congeners against herpes simplex virus (HSV) [14] (Fig. 1).

Because of these unexpected results for such highly modified nucleoside analogs, we investigated other guanosine derivatives that target viruses of major public health concern such as HIV and hepatitis C virus (HCV). HCV has infected an estimated 170 million individuals worldwide and is a major cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma. Currently the only approved therapy for chronic HCV infection is pegylated interferon-α in combination with ribavirin, a modality often poorly tolerated and effective in only half of the genotype 1 population [15,16]. On the other hand an estimated 33 million people worldwide live with HIV. In 2007, it is estimated HIV killed an estimated 2.1 million people, including 330,000 children. Although treatments for HIV can slow the course of the disease, there is currently no vaccine or cure. For both HIV and HCV there is significant need for new and more effective therapies. Based on the literature [17,18] and our own antiviral expertise, we investigated the influence of this tricyclic modification on 9-[4-α-(hydroxymethyl)-cyclopent-2-ene-1-α-yl]guanine (CBV) **5**, (−)-β-D-(2*R*,4*R*)-1,3-dioxolane-guanosine (DXG) **6**, 3′-azido-3′-deoxyguanosine (AZG) **7**, and 2′-*C*-methyl-guanosine **8** all well known for their anti-HIV [19] or anti-HCV activities [20–23] (Fig. 2).

2. Results and discussion

2.1. Chemistry

CBV **5** [24], DXG **6**, AZG **7** [25], and 2′-*C*-methylguanosine **8** [26], were synthesized according to literature procedures (Fig. 2). The corresponding tricyclic derivatives were prepared by reacting nucleoside analogs **5**–**8** with an appropriate bromoketone in the presence of sodium hydride following the previously reported

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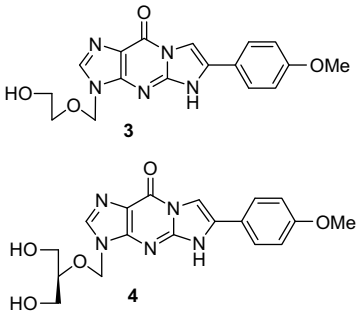


Fig. 1. Structure of tricyclic derivatives of ACV and GCV.

method for this alkylation–condensation reaction (Scheme 2). All the synthesized tricyclic nucleoside analogs, **9**–**40**, have been characterized by ^{1}H NMR while their purity and nominal mass were checked by LC/MS before biological evaluation.

2.2. Antiviral evaluation and discussion

From our SAR effort (Table 1), several observations can be made. First, the antiviral selectivity seemed to be related to the structure of the sugar moiety. Indeed, the tricyclic compounds **36**–**39** with a 2′-*C*-methylribo sugar showed selective anti-HCV activity, like its parent nucleoside **8**. On the other hand, compounds **9**–**34** synthesized from HIV active compound CBV **5**, DXG **6** and AZG **7** showed selective anti-HIV activity. The second important point is that antiviral activities correlate with the electron richness of the annulated aryl group of the tricyclic derivative. Thus, compounds bearing an electron donor group such as 4-MeO-Ph (**36**), 4-NEt_2-Ph (**37**), 4-NMe_2-Ph (**38**), 2-thiophenyl (**39**) showed EC_{50} values against HCV below 10 μM. In the same manner, tricyclic compounds obtained from CBV, DXG, and AZG with a 2-thiophenyl (**14**, **27**), 4-NEt_2-Ph (**12**, **25**, **33**), 4-NMe_2-Ph (**13**, **26**, **34**), 4-MeO-Ph (**9**, **18**, **32**) group possessed anti-HIV activity similar to their corresponding parent nucleoside analogs. However, other substitutions such as ethyl (**16**, **35**), 4-CN-Ph (**30**) or 4-Cl-Ph (**22**) showed less or no activity in all cases. The influence of the electronic properties of the substitution observed in our case seems to be in accordance with the literature since electron withdrawing substituents such as 4-NO_2-Ph, naphtalenyl, 3-MeO-Ph or 4-CF_3-Ph are not active in the ACV/GCV series [14]. Finally, it is noteworthy that the most potent compounds in each series demonstrated a level of potency similar to the parent nucleoside.

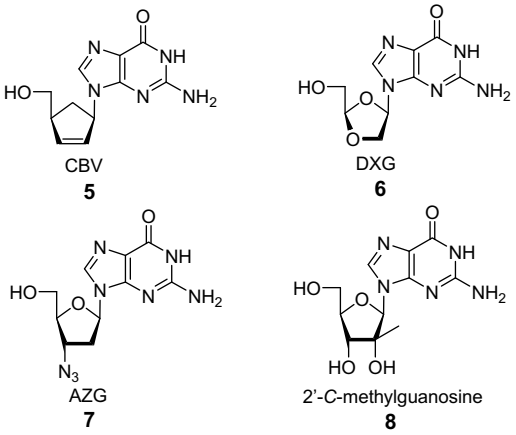
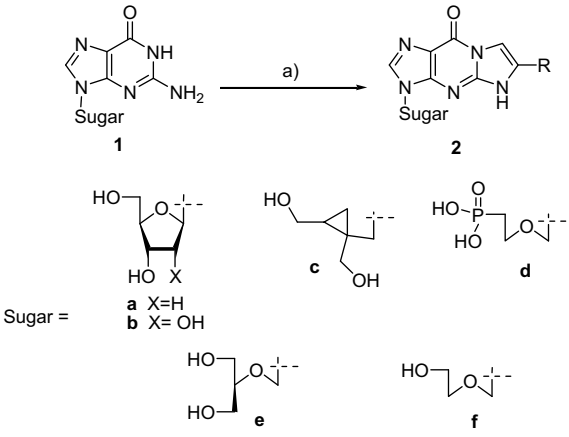


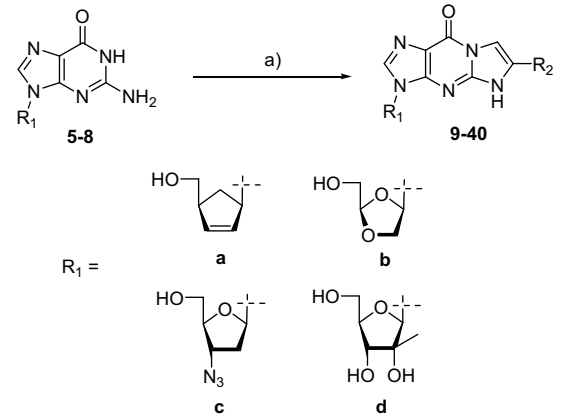
Fig. 2. Structure of CBV, DXG, AZG and 2′-*C*-methylguanosine.



Scheme 1. (a) NaH, $BrCH_2(CO)R$, DMF, rt.

The similarities of antiviral profiles between parent nucleosides and corresponding tricyclic nucleoside, also observed with ACV and GCV derivatives, lead us to question the stability of the electron rich tricycles in cells or in solution. Indeed, several groups have suggested that this kind of tricycle might serve as a prodrug of the parent nucleoside [5] however; no data has ever been generated to support this hypothesis.

Since nucleosides are prodrugs that require conversion to the corresponding triphosphate anabolite *via* cellular kinases, the cellular level of phosphorylation for compound **38**, which showed significant activity against HCV, was evaluated. However, after incubation of compound **38** at 50 μM in Huh-7 cells for 4 h no tricyclic-monophosphate (MP), -diphosphate (DP), -triphosphate (TP) or even simple compound **38** were detected by LC–MS/MS. On the other hand, significant levels of the parents compound **8** and its MP, DP and TP were detected intra and extracellular, indicating that the observed activity does not come from the triphosphate of **38**, but from conversion to **8-TP**. To confirm these results, the stability of various tricyclic compounds in solution was studied using LC–MS/MS for detection of potential products. Compound **33**, one of the most potent compound against HIV, was unstable in water with a half-life of 25 min. However, the same compound **33** was more stable in MeOH with only 1% conversion to **7** after 72 h. Compounds **32**, **36** and **39** which displayed lower antiviral activity also reverted to parent nucleoside at slower rates (respectively 60% of **7**, 30% of **8** and 60% of **8** after 3 weeks). These differences in the rate of decomposition could certainly explain the variations of activity in



Scheme 2. (a) NaH, $BrCH_2(CO)R$, DMF, rt, 10–50%.

Table 1
In vitro anti-HCV activity, anti-HIV activity and cellular toxicity of nucleoside analogs **9**–**40**. nd: not determined.

Compound	R_1	R_2	Anti-HIV activity in human PBM cells (μM)		Anti-HCV activity Huh-7 cells (μM)		Cytotoxicity (CC_{50} in μM)		
			EC_{50}	EC_{90}	EC_{50}	EC_{90}	PBM	CEM	VERO
CBV, **5**			0.043	0.29	>10	>10	7.0	>100	>100
9	**a**	4-MeO-Ph	0.62	4.9	>10	>10	>100	>100	>100
10	**a**	4-Me-Ph	5.0	30.1	>10	>10	>100	>100	>100
11	**a**	4-Br-Ph	12.3	43.2	>10	>10	>100	>100	>100
12	**a**	4-NEt_2-Ph	0.13	0.65	>10	>10	>100	>100	>100
13	**a**	4-NMe_2-Ph	0.11	0.47	>10	>10	24.0	>100	>100
14	**a**	2-thiophenyl	0.64	7.1	>10	>10	>100	>100	>100
15	**a**	3-thiophenyl	2.6	15.5	>10	>10	>100	>100	>100
DXG, **6**			0.51	2.4	>10	>10	>100	>100	>100
16	**b**	Et	31.5	>100	>10	>10	>100	>100	>100
17	**b**	Ph	7.1	43.5	>10	>10	>100	>100	>100
18	**b**	4-MeO-Ph	3.45	23.9	>10	>10	>100	>100	>100
19	**b**	3-MeO-Ph	20.7	89.6	>10	>10	>100	>100	>100
20	**b**	2-MeO-Ph	8.5	26.8	>10	>10	>100	>100	>100
21	**b**	4-Me-Ph	4.9	23.9	>10	>10	>100	>100	>100
22	**b**	4-Cl-Ph	>100	>100	>10	>10	>100	>100	>100
23	**b**	4-F-Ph	43.5	>100	>10	>10	>100	>100	>100
24	**b**	2,4-F-Ph	14.7	51.8	>10	>10	>100	>100	>100
25	**b**	4-NEt_2-Ph	0.25	1.25	>10	>10	>100	80.8	63.0
26	**b**	4-NMe_2-Ph	0.61	1.5	>10	>10	>100	>100	>100
27	**b**	2-thiophenyl	3.0	16	>10	>10	>100	>100	>100
28	**b**	3-thiophenyl	0.91	6.6	>10	>10	>100	>100	>100
29	**b**	4-N_3-Ph	2.6	11.4	>10	>10	>100	>100	>100
30	**b**	4-CN-Ph	69.3	>100	>10	>10	>100	>100	>100
AZG, **7**			0.18	1.98	>10	>10	>100	>100	>100
31	**c**	Ph	8.4	94.1	>10	>10	69.6	21.8	>100
32	**c**	4-MeO-Ph	1.3	7.0	>10	>10	56.7	13.5	>100
33	**c**	4-NEt_2-Ph	0.04	0.34	>10	>10	>100	14.1	72.8
34	**c**	4-NMe_2-Ph	0.14	0.54	>10	>10	>100	74.6	>100
2′-*C*-MeG, **8**			>100	>100	2.3	7.8	>100	>100	>100
35	**d**	Et	>100	>100	>10	>10	>100	>100	>100
36	**d**	4-MeO-Ph	>100	>100	≥10	>10	>100	>100	>100
37	**d**	4-NEt_2-Ph	>100	>100	≤10	nd	>100	>100	>100
38	**d**	4-NMe_2-Ph	>100	>100	4.4	9.5	>100	>100	>100
39	**d**	2-Thiophenyl	>100	>100	6.9	25.6	>100	>100	44.3
40	**d**	3-Thiophenyl	>100	>100	≥10	>10	>100	>100	>100

each series. A more stable tricyclic compound will more slowly convert to its active parent and conversely, a less stable tricyclic compound will generate more of its active nucleoside parent.

With these surprising stability results, we decided to turn our attention on to the tricyclic ACV analog 6-(4-MeOPh)-TACV **3**, which, according to the literature, shows a similar potency and selectivity against HSV-1 and HSV-2 as ACV [14]. Compound **3** was synthesized following the published procedure and evaluated for activity against HSV-1. First of all, **3** showed some activity against HSV-1 ($EC_{50} = 12.2$ μM, $EC_{90} = 23.7$ μM) even though **3** appears less potent than ACV in our assay ($EC_{50} = 0.075$ μM, $EC_{90} = 0.7$ μM) and less potent than what has been reported. Using the method previously described, the stability of compound **3** in water was studied and we found that it slowly converted to ACV (4% after 4 days and 35% after 3 weeks).

3. Conclusions

Despite the abundance of literature concerning this type of tricyclic nucleosides, we are the first to report the instability of some 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*A*]purine nucleosides in aqueous media. These results indicate that the activity of the electron rich tricyclic nucleosides was derived from their ability to convert to the active parent nucleoside, thus acting as prodrugs. More studies will be needed in order to determine if the differences in solubility and polarity of these electron rich tricyclic nucleosides translate into any advantage for the *in vivo* delivery of therapeutically important nucleoside analogs.

4. Experimental procedure

4.1. Synthesis

Anhydrous solvents were purchased from Aldrich Chemical Company, Inc. Reagents were purchased from commercial sources. Unless noted otherwise, the materials used in the examples were obtained from readily available commercial suppliers or synthesized by standard methods known to one skilled in the art of chemical synthesis. ^{1}H NMR spectra were taken on a Varian Unity Plus 400 spectrometer at room temperature and reported in parts per million downfield from internal tetramethylsilane. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quadruplet), br (broad), bs (broad singlet), m (multiplet). All *J* values are in hertz. Mass spectral analyses were performed on a Micromass TOF instrument (Hewlett-Packard HPLC driven electrospray MS instrument). Analytical HPLC analyses were performed on a Hewlett-Packard HPLC with a Phenomenex Gemini-NX column (2 mm × 50 mm, 3 μm, C18, 110 Å). Mobile phase flow was 0.7 ml/min with a 3.5 min gradient from 96% aqueous media (0.05% formic acid) to 96% CH_3CN (0.05% formic acid) with a 5.5 min total acquisition time and 190–360 nm PDA detection.

4.1.1. General procedure for the synthesis of compounds ***9–40***

To a solution of **5**–**8** (1 equiv) in anhydrous DMF (20 ml/1 mmol of **5**–**8**) was added sodium hydride as 60% suspension in oil (1.1 equiv). After being stirred for 2 h at room temperature, the

resulting solution was treated with bromoketone (1.3 equiv) and stirred overnight. After methanolysis, the volatiles were evaporated and the residual solid was chromatographed on silica gel column using CH_2Cl_2–MeOH (9:1 or 8:2). If necessary, the crude material, after chromatography, was then washed with a minimum volume of MeOH to afford the desired compounds **9**–**40**.

*4.1.1.1. 3-[4-(Hydroxymethyl)-2-cyclopenten-1-yl]-3,9-dihydro-6-(4-methoxyphenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**9**).* ^{1}H NMR (DMSO-d_6) δ 1.58–1.64 (m, 1H), 2.58–2.66 (m, 1H), 2.83–2.87 (m, 1H), 3.39–3.43 (m, 2H), 3.76 (s, 3H, CH_3), 4.73 (br s, 1H), 5.43–5.47 (m, 1H), 5.91–5.93 (m, 1H), 6.12–6.14 (m, 1H), 6.98 (d, 2H, $J = 8.8$ Hz), 7.71 (s, 1H), 7.80 (d, 2H, $J = 8.8$ Hz), 7.95 (s, 1H), 12.91 (br s, NH); ^{13}C NMR (DMSO-d_6) δ 34.8, 48.5, 55.9, 59.6, 64.7, 102.5, 115.1, 116.4, 121.1, 127.2, 129.6, 130.1, 137.3, 139.3, 146.9, 150.3, 152.0, 160.3; LC–MS Calcd for $C_{20}H_{18}N_5O_3$ 377.4; observed (M + 1) 378.3.

*4.1.1.2. 3-[4-(Hydroxymethyl)-2-cyclopenten-1-yl]-3,9-dihydro-6-(4-methylphenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**10**).* ^{1}H NMR (DMSO-d_6) δ 1.58–1.65 (m, 1H), 2.30 (s, 3H, CH_3), 2.58–2.66 (m, 1H), 2.83–2.87 (m, 1H), 3.39–3.43 (m, 2H), 4.72 (br s, 1H), 5.43–5.47 (m, 1H), 5.91–5.94 (m, 1H), 6.12–6.15 (m, 1H), 7.24 (d, 2H, $J = 7.6$ Hz), 7.74–7.78 (m, 3H), 8.09 (s, 1H), 12.90 (br s, NH). LC–MS Calcd for $C_{20}H_{19}N_5O_3$ 361.4; observed (M + 1) 362.5.

*4.1.1.3. 3-[4-(Hydroxymethyl)-2-cyclopenten-1-yl]-3,9-dihydro-6-(4-bromophenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**11**).* ^{1}H NMR (DMSO-d_6) δ 1.58–1.65 (m, 1H), 2.59–2.67 (m, 1H), 2.83–2.87 (m, 1H), 3.39–3.43 (m, 2H), 4.69–4.73 (m, 1H), 5.43–5.47 (m, 1H), 5.92–5.94 (m, 1H), 6.13–6.15 (m, 1H), 7.65 (d, 2H, $J = 8.8$ Hz), 7.79–7.85 (m, 3H), 8.26 (s, 1H), 13.01 (br s, NH). LC–MS Calcd for $C_{19}H_{16}BrN_5O_2$ 425.3/427.4; observed (M + 1) 426.1/428.2.

*4.1.1.4. 3-[4-(Hydroxymethyl)-2-cyclopenten-1-yl]-3,9-dihydro-6-(4-diethylaminophenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**12**).* ^{1}H NMR (DMSO-d_6) δ 1.03–1.07 (m, 6H, 2 × CH_3), 1.58–1.65 (m, 1H), 2.57–2.66 (m, 1H), 2.83–2.87 (m, 1H), 3.31–3.36 (m, 4H, 2 × CH_2), 3.39–3.43 (m, 2H, CH_2), 4.72 (br s, 1H), 5.43–5.47 (m, 1H), 5.91–5.94 (m, 1H), 6.12–6.15 (m, 1H), 6.67 (d, 2H, $J = 8.0$ Hz), 7.63–7.68 (m, 3H), 7.85 (s, 1H), 12.82 (br s, NH). LCMS Calcd for $C_{23}H_{26}N_6O_2$ 418.5; observed (M + 1) 419.4.

*4.1.1.5. 3-[4-(Hydroxymethyl)-2-cyclopenten-1-yl]-3,9-dihydro-6-(4-dimethylaminophenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**13**).* ^{1}H NMR (DMSO-d_6) δ 1.58–1.65 (m, 1H), 2.58–2.66 (m, 1H), 2.83–2.87 (m, 1H), 2.92 (s, 6H, 2 × CH_3), 3.39–3.43 (m, 2H), 4.71 (t, 1H, $J = 5.6$ Hz), 5.43–5.47 (m, 1H), 5.91–5.94 (m, 1H), 6.12–6.15 (m, 1H), 6.74 (d, 2H, $J = 8.8$ Hz), 7.67 (d, 2H, $J = 8.8$ Hz), 7.77 (s, 1H), 7.88 (s, 1H), 12.8 (br s, NH). LCMS Calcd for $C_{21}H_{22}N_6O_2$ 390.4; observed (M + 1) 391.4.

*4.1.1.6. 3-[4-(Hydroxymethyl)-2-cyclopenten-1-yl]-3,9-dihydro-6-(2-thienyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**14**).* ^{1}H NMR (DMSO-d_6) δ 1.59–1.65 (m, 1H), 2.58–2.66 (m, 1H), 2.83–2.87 (m, 1H), 3.39–3.43 (m, 2H, CH_2), 4.71 (br s, 1H), 5.43–5.47 (m, 1H), 5.91–5.94 (m, 1H), 6.12–6.14 (m, 1H), 7.12–7.15 (m, 1H), 7.57–7.58 (m, 1H), 7.62 (d, 1H, $J = 5.2$ Hz), 7.79 (s, 1H), 7.88 (s, 1H), 13.19 (br s, NH). LCMS Calcd for $C_{17}H_{15}N_5O_2S$ 353.4; observed (M + 1) 354.5.

*4.1.1.7. 3-[4-(Hydroxymethyl)-2-cyclopenten-1-yl]-3,9-dihydro-6-(3-thienyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**15**).* ^{1}H NMR (DMSO-d_6) δ 1.59–1.65 (m, 1H), 2.58–2.64 (m, 1H), 2.83–2.87 (m, 1H), 3.39–3.43 (m, 2H, CH_2), 4.70–4.73 (m, 1H), 5.44–5.47 (m, 1H), 5.92–5.95 (m, 1H), 6.12–6.15 (m, 1H), 7.67–7.68 (m, 2H), 7.79 (s, 1H), 7.95–7.96 (m, 1H), 8.08 (s, 1H), 13.00 (br s, NH). LCMS Calcd for $C_{17}H_{15}N_5O_2S$ 353.4; observed (M + 1) 354.5.

*4.1.1.8. 3-(β-D-1,3-Dioxolanyl)-3,9-dihydro-6-ethyl-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**16**).* ^{1}H NMR (DMSO-d_6) δ 1.2 (t, 3H, $J = 7.6$ Hz, CH_3), 2.55–2.61 (m, 2H, CH_2), 3.41–3.29 (m, 2H, CH_2), 4.16–4.21 (m, 1H), 4.45–4.48 (m, 1H), 5.01–5.02 (m, 1H), 5.12–5.15 (m, 1H), 6.26 (d, 1H, $J = 5.2$ Hz), 7.33 (s, 1H), 8.00 (s, 1H), 8.27 (s, 1H), 13.01 (br s, NH). LCMS Calcd for $C_{17}H_{15}N_5O_2S$ 305.3; observed (M + 1) 306.4.

*4.1.1.9. 3-(β-D-1,3-Dioxolanyl)-3,9-dihydro-6-phenyl-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**17**).* ^{1}H NMR (DMSO-d_6) δ 3.57–3.60 (m, 2H, CH_2), 4.19–4.24 (m, 1H), 4.50–4.53 (m, 1H), 5.04 (t, 1H, $J = 2.8$ Hz), 5.14 (t, 1H, $J = 6.0$ Hz), 6.29 (d, 1H, $J = 4.0$ Hz), 7.34–7.38 (m, 1H), 7.43–7.47 (m, 2H), 7.88 (d, 2H, $J = 7.2$ Hz), 8.06 (s, 1H), 8.20 (s, 1H), 13.05 (br s, NH). LCMS Calcd for $C_{17}H_{15}N_5O_5$ 353.3; observed (M + 1) 354.4.

*4.1.1.10. 3-(β-D-1,3-Dioxolanyl)-2-cyclopenten-1-yl]-3,9-dihydro-6-(4-methoxyphenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**18**).* ^{1}H NMR (DMSO-d_6) δ 3.56–3.60 (m, 2H, CH_2), 3.77 (s, 3H, CH_3), 4.19–4.24 (m, 1H), 4.49–4.53 (m, 1H), 5.02–5.05 (m, 1H), 5.12–5.15 (m, 1H), 6.29 (d, 1H, $J = 4.8$ Hz), 7.01 (d, 2H, $J = 8.8$ Hz), 7.81 (d, 2H, $J = 8.8$ Hz), 8.05 (s, 1H), 8.06 (s, 1H), 12.98 (br s, NH); ^{13}C NMR (DMSO-d_6) δ 56.0, 61.8, 71.2, 79.9, 102.6, 106.2, 115.1, 115.9, 120.9, 127.2, 129.8, 137.0, 146.3, 150.3, 151.9, 160.3; LCMS Calcd for $C_{18}H_{17}N_5O_5$ 383.4; observed (M + 1) 384.5.

*4.1.1.11. 3-(β-D-1,3-Dioxolanyl)-3,9-dihydro-6-(3-methoxyphenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**19**).* ^{1}H NMR (DMSO-d_6) δ 3.56–3.60 (m, 2H, CH_2), 3.80 (s, 3H, CH_3), 4.19–4.24 (m, 1H), 4.49–4.53 (m, 1H), 5.03–5.05 (m, 1H), 5.12–5.15 (m, 1H), 6.29 (d, 1H, $J = 4.8$ Hz), 6.92 (d, 1H, $J = 7.2$ Hz), 7.32–7.37 (m, 1H), 7.45–7.48 (m, 2H), 8.05 (s, 1H), 8.27 (s, 1H), 13.05 (br s, NH). LCMS Calcd for $C_{18}H_{17}N_5O_5$ 383.4; observed (M + 1) 384.4.

*4.1.1.12. 3-(β-D-1,3-Dioxolanyl)-3,9-dihydro-6-(2-methoxyphenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**20**).* ^{1}H NMR (DMSO-d_6) δ 3.56–3.60 (m, 2H, CH_2), 3.95 (s, 3H, CH_3), 4.19–4.23 (m, 1H), 4.49–4.51 (m, 1H), 5.03–5.05 (m, 1H), 5.10–5.13 (m, 1H), 6.29 (d, 1H, $J = 3.6$ Hz), 7.03–7.07 (m, 1H), 7.15–7.17 (m, 1H), 7.34–7.39 (m, 1H), 7.82–7.84 (m, 1H), 7.92 (s, 1H), 8.04 (s, 1H), 13.05 (br s, NH). LCMS Calcd for $C_{18}H_{17}N_5O_5$ 383.4; observed (M + 1) 384.4.

*4.1.1.13. 3-(β-D-1,3-Dioxolanyl)-3,9-dihydro-6-(4-methylphenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**21**).* ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H, CH_3), 3.56–3.60 (m, 2H, CH_2), 4.19–4.23 (m, 1H), 4.47–4.50 (m, 1H), 5.03–5.04 (m, 1H), 5.14–5.17 (m, 1H), 6.29 (d, 1H, $J = 4.4$ Hz), 7.24 (d, 2H, $J = 8.4$ Hz), 7.75 (d, 2H, $J = 8.4$ Hz), 8.00 (s, 1H), 8.07 (s, 1H), 13.02 (br s, NH). LCMS Calcd for $C_{18}H_{17}N_5O_4$ 367.4; observed (M + 1) 368.5.

*4.1.1.14. 3-(β-D-1,3-Dioxolanyl)-3,9-dihydro-6-(4-chlorophenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**22**).* ^{1}H NMR (DMSO-d_6) δ 3.55–3.60 (m, 2H, CH_2), 4.19–4.22 (m, 1H), 4.47–4.49 (m, 1H), 5.03–5.04 (m, 1H), 5.14–5.19 (m, 1H), 6.29 (d, 1H, $J = 4.4$ Hz), 7.48 (d, 2H, $J = 8.4$ Hz), 7.89 (d, 2H, $J = 8.4$ Hz), 7.98 (s, 1H), 8.17 (s, 1H), 13.04 (br s, NH). LCMS Calcd for $C_{17}H_{14}ClN_5O_4$ 387.8; observed (M + 1) 388.1/390.2.

*4.1.1.15. 3-(β-D-1,3-Dioxolanyl)-3,9-dihydro-6-(4-fluorophenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**23**).* ^{1}H NMR (DMSO-d_6) δ 3.57–3.60 (m, 2H, CH_2), 4.19–4.23 (m, 1H), 4.51 (d, 1H, $J = 9.2$ Hz), 5.03–5.05 (m, 1H), 5.12 (t, 1H, $J = 6.0$ Hz), 6.29 (d, 1H, $J = 4.4$ Hz), 7.29–7.33

(m, 2H), 7.91–7.95 (m, 2H), 8.05 (s, 1H), 8.21 (s, 1H), 13.09 (br s, NH). LCMS Calcd for $C_{17}H_{14}N_5O_4$ 371.3; observed (M + 1) 372.2.

*4.1.1.16. 3-(β-D-1,3-Dioxolanyl)-3,9-dihydro-6-(2,4-difluorophenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**24**).* ^{1}H NMR (DMSO-d_6) δ 3.56–3.59 (m, 2H, CH_2), 4.19–4.23 (m, 1H), 4.50 (d, 1H, J = 9.2 Hz), 5.03–5.05 (m, 1H), 5.14 (br s, 1H), 6.29–6.31 (m, 1H), 7.24–7.29 (m, 1H), 7.43–7.49 (m, 1H), 7.81 (d, 1H, J = 3.2 Hz), 7.92–7.98 (m, 1H), 8.03 (s, 1H), 13.01 (br s, NH). LCMS Calcd for $C_{17}H_{13}F_2N_5O_4$ 389.3; observed (M + 1) 390.3.

*4.1.1.17. 3-(β-D-1,3-Dioxolanyl)-3,9-dihydro-6-(4-diethylaminophenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**25**).* ^{1}H NMR (DMSO-d_6) δ 1.07 (t, 6H, J = 6.8 Hz, 2 × CH_3), 3.27–3.37 (m, 4H, 2 × CH_2), 3.57–3.60 (m, 2H, CH_2), 4.18–4.23 (m, 1H), 4.49–4.52 (m, 1H), 5.03–5.04 (m, 1H), 5.11 (t, 1H, J = 6.0 Hz), 6.28 (d, 1H, J = 4.8 Hz), 6.68 (d, 2H, J = 8.8 Hz), 7.64 (d, 2H, J = 8.8 Hz), 7.86 (s, 1H), 8.03 (s, 1H), 12.81 (br s, NH). LCMS Calcd for $C_{21}H_{24}N_6O_4$ 424.5; observed (M + 1) 425.6.

*4.1.1.18. 3-(β-D-1,3-Dioxolanyl)-3,9-dihydro-6-(4-dimethylaminophenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**26**).* ^{1}H NMR (DMSO-d_6) δ 2.92 (s, 6H, 2 × CH_3), 3.57–3.60 (m, 2H, CH_2), 4.19–4.23 (m, 1H), 4.49–4.52 (m, 1H), 5.03–5.04 (m, 1H), 5.12 (t, 1H, J = 6.0 Hz), 6.28 (d, 1H, J = 4.8 Hz), 6.74 (d, 2H, J = 8.8 Hz), 7.68 (d, 2H, J = 8.8 Hz), 7.90 (s, 1H), 8.02 (s, 1H), 12.84 (br s, NH). LCMS Calcd for $C_{19}H_{20}N_6O_4$ 396.4; observed (M + 1) 397.3.

*4.1.1.19. 3-(β-D-1,3-Dioxolanyl)-3,9-dihydro-6-(2-thienyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**27**).* ^{1}H NMR (DMSO-d_6) δ 3.56–3.60 (m, 2H, CH_2), 4.19–4.23 (m, 1H), 4.49–4.51 (m, 1H), 5.02–5.04 (m, 1H), 5.12 (t, 1H, J = 6.4 Hz), 6.28 (d, 1H, J = 4.4 Hz), 7.13–7.15 (m, 1H), 7.58 (d, 1H, J = 3.2 Hz), 7.62–7.64 (m, 1H), 7.91 (s, 1H), 8.04 (s, 1H), 13.17 (br s, NH). LCMS Calcd for $C_{15}H_{13}N_5O_4S$ 359.4; observed (M + 1) 360.5.

*4.1.1.20. 3-(β-D-1,3-Dioxolanyl)-3,9-dihydro-6-(3-thienyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**28**).* ^{1}H NMR (DMSO-d_6) δ 3.57–3.60 (m, 2H, CH_2), 4.19–4.23 (m, 1H), 4.49–4.52 (m, 1H), 5.02–5.04 (m, 1H), 5.12–5.15 (m, 1H), 6.29 (d, 1H, J = 5.6 Hz), 7.67–7.68 (m, 2H), 7.97 (s, 1H), 8.04 (s, 1H), 8.10 (s, 1H), 13.06 (br s, NH). LCMS Calcd for $C_{15}H_{18}N_5O_4S$ 359.4; observed (M + 1) 360.3.

*4.1.1.21. 3-(β-D-1,3-Dioxolanyl)-3,9-dihydro-6-(4-azido-phenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**29**).* ^{1}H NMR (DMSO-d_6) δ 3.56–3.60 (m, 2H, CH_2), 4.19–4.23 (m, 1H), 4.51 (d, 1H, J = 9.2 Hz), 5.00–5.03 (m, 1H), 5.12 (t, 1H, J = 6.0 Hz), 6.28 (d, 1H, J = 4.8 Hz), 7.19 (d, 2H, J = 8.4 Hz), 7.91 (d, 2H, J = 8.4 Hz), 8.04 (s, 1H), 8.20 (s, 1H), 13.07 (br s, NH). LCMS Calcd for $C_{17}H_{14}N_8O_4$ 394.3; observed (M + 1) 395.4.

*4.1.1.22. 3-(β-D-1,3-Dioxolanyl)-3,9-dihydro-6-(4-cyano-phenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**29**).* ^{1}H NMR (DMSO-d_6) δ 3.57–3.60 (m, 2H, CH_2), 4.19–4.24 (m, 1H), 4.50–4.53 (m, 1H), 5.03–5.04 (m, 1H), 5.11–5.15 (m, 1H), 6.29 (d, 1H, J = 4.4 Hz), 7.91–7.93 (m, 2H), 8.05–8.08 (m, 3H), 8.47 (s, 1H), 13.25 (br s, NH). LCMS Calcd for $C_{18}H_{14}N_6O_4$ 378.3; observed (M + 1) 379.2.

*4.1.1.23. 3-(3-Azido-2,3-dideoxy-β-D-erythro-pentofuranosyl)-3,9-dihydro-6-phenyl-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**31**).* ^{1}H NMR (DMSO-d_6) δ 2.46–2.52 (m, 1H), 2.82–2.89 (m, 1H), 3.53–3.61 (m, 2H, CH_2), 3.87–3.90 (m, 1H), 4.52–4.59 (m, 1H), 5.12–5.16 (m, 1H), 6.21 (t, 1H, J = 6.0 Hz), 7.35–7.39 (m, 1H), 7.43–7.47 (m, 2H), 7.87–7.89 (m, 2H), 8.16 (s, 1H), 8.20 (s, 1H), 13.02 (br s, NH). LCMS Calcd for $C_{18}H_{16}N_8O_3$ 392.4; observed (M + 1) 393.3.

*4.1.1.24. 3-(3-Azido-2,3-dideoxy-β-D-erythro-pentofuranosyl)-3,9-dihydro-6-(4-methoxyphenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**32**).* ^{1}H NMR (DMSO-d_6) δ 2.46–2.52 (m, 1H), 2.83–2.90 (m, 1H), 3.53–3.61 (m, 2H, CH_2), 3.77 (s, 3H, OCH_3), 3.88–3.90 (m, 1H), 4.54–4.59 (m, 1H), 5.11–5.17 (m, 1H), 6.21 (t, 1H, J = 6.4 Hz), 7.01 (d, 2H, J = 8.6 Hz), 7.81 (d, 2H, J = 8.6 Hz), 8.06 (s, 1H), 8.15 (s, 1H), 12.93 (br s, NH). ^{13}C NMR (DMSO-d_6) δ 36.9, 55.9, 61.4, 61.8, 83.2, 85.2, 102.6, 115.1, 116.4, 121.0, 127.2, 129.8, 137.5, 146.8, 150.2, 151.9, 160.3; LCMS Calcd for $C_{19}H_{18}N_8O_4$ 422.4; observed (M + 1) 423.5.

*4.1.1.25. 3-(3-Azido-2,3-dideoxy-β-D-erythro-pentofuranosyl)-3,9-dihydro-6-(4-diethylaminophenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**33**).* ^{1}H NMR ($CDCl_3$) δ 1.06–1.25 (m, 6H, 2 × CH_3), 2.30–2.35 (m, 1H), 3.10–3.15 (m, 1H), 3.22–3.29 (m, 2H, CH_2), 3.77 (d, 1H, J = 12.4 Hz), 4.06 (d, 1H, J = 12.4 Hz), 4.19 (s, 1H), 4.44–4.46 (m, 1H), 6.10–6.14 (m, 1H), 6.58 (d, 2H, J = 8.6 Hz), 7.36 (d, 2H, J = 8.6 Hz), 7.55 (s, 1H), 7.65 (s, 1H). LCMS Calcd for $C_{22}H_{25}N_9O_3$ 463.5; observed (M + 1) 464.6.

*4.1.1.26. 3-(3-Azido-2,3-dideoxy-β-D-erythro-pentofuranosyl)-3,9-dihydro-6-(4-dimethylaminophenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**34**).* ^{1}H NMR (DMSO-d_6) δ 2.81–3.02 (m, 8H, 2 × CH_3, CH_2), 3.53–3.61 (m, 2H, CH_2), 3.87–3.90 (m, 1H), 4.52–4.59 (m, 1H), 5.11–5.17 (m, 1H), 6.18–6.23 (m, 1H), 6.75 (d, 2H, J = 8.6 Hz), 7.68 (d, 2H, J = 8.6 Hz), 7.91 (s, 1H), 8.13 (s, 1H), 12.85 (br s, NH). LCMS Calcd for $C_{20}H_{21}N_9O_3$ 435.5; observed (M + 1) 436.5.

*4.1.1.27. 3-(3-azido-2,3-dideoxy-β-D-erythro-pentofuranosyl)-3,9-dihydro-6-ethyl-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**35**).* ^{1}H NMR (DMSO-d_6) δ 0.79 (s, 3H, CH_3), 1.19 (t, 3H, J = 7.6 Hz, CH_3), 2.56–2.60 (m, 2H, CH_2), 3.62–3.67 (m, 1H), 3.77–3.86 (m, 2H), 3.95–4.00 (m, 1H), 5.10 (s, OH), 5.15–5.17 (m, OH), 5.25 (d, J = 6.8 Hz, OH), 5.82 (s, 1H, H-1′), 7.31 (s, 1H), 8.21 (s, 1H), 12.91 (br s, NH). LCMS Calcd for $C_{15}H_{19}N_5O_5$ 349.3; observed (M + 1) 350.2.

*4.1.1.28. 3-(β-D-2-C-Methyl-ribofuranosyl)-3,9-dihydro-6-(4-methoxyphenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**36**).* ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H, CH_3), 3.62–3.70 (m, 1H), 3.77–3.88 (m, 4H), 3.96–4.00 (m, 1H), 4.06–4.10 (m, 1H), 5.10 (s, OH), 5.17–5.19 (m, OH), 5.29 (d, J = 6.4 Hz, OH), 5.86 (s, 1H, H-1′), 7.00 (d, 2H, J = 8.8 Hz), 7.81 (d, 2H, J = 8.8 Hz), 8.05 (s, 1H), 8.25 (s, 1H), 13.05 (br s, NH); ^{13}C NMR (DMSO-d_6) δ 20.7, 55.9, 60.0, 72.2, 79.3, 83.0, 91.2, 102.5, 115.2, 116.1, 121.0, 127.2, 129.8, 137.2, 146.9, 150.2, 151.9, 160.3; LCMS Calcd for $C_{20}H_{21}N_5O_5$ 427.4; observed (M + 1) 428.3.

*4.1.1.29. 3-(β-D-2-C-Methyl-ribofuranosyl)-3,9-dihydro-6-(4-diethylaminophenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**37**).* ^{1}H NMR (DMSO-d_6) δ 0.79 (s, 3H, CH_3), 1.00–1.13 (m, 6H, 2 × CH_3), 3.33–3.40 (m, 4H, 2 × CH_2), 3.63–3.68 (m, 1H), 3.78–3.87 (m, 2H), 3.95–4.00 (m, 1H), 5.07 (s, OH), 5.17–5.19 (m, OH), 5.28 (d, J = 6.8 Hz, OH), 5.86 (s, 1H, H-1′), 6.67 (d, 2H, J = 8.8 Hz), 7.63 (d, 2H, J = 8.8 Hz), 7.84 (s, 1H), 8.22 (s, 1H), 12.85 (br s, NH). LCMS Calcd for $C_{23}H_{28}N_6O_5$ 468.5; observed (M + 1) 469.6.

*4.1.1.30. 3-(β-D-2-C-Methyl-ribofuranosyl)-3,9-dihydro-6-(4-dimethylaminophenyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**38**).* ^{1}H NMR (DMSO-d_6) δ 0.79 (s, 3H, CH_3), 2.92 (s, 6H, 2 × CH_3), 3.63–3.68 (m, 1H), 3.79–3.97 (m, 2H), 3.96–4.01 (m, 1H), 5.08 (s, OH), 5.17–5.19 (m, OH), 5.28 (d, J = 6.8 Hz, OH), 5.86 (s, 1H, H-1′), 6.74 (d, 2H, J = 8.8 Hz), 7.67 (d, 2H, J = 8.8 Hz), 7.90 (s, 1H), 8.25 (s, 1H), 12.89 (br s, NH). LCMS Calcd for $C_{21}H_{24}N_6O_5$ 440.4; observed (M + 1) 441.5.

*4.1.1.31. 3-(β-D-2-C-Methyl-ribofuranosyl)-3,9-dihydro-6-(2-thienyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**39**).* ^{1}H NMR (DMSO-d_6) δ 0.80 (s, 3H, CH_3), 3.64–3.68 (m, 1H), 3.79–3.87 (m, 2H), 3.96–4.00 (m, 1H),

5.09 (s, OH), 5.17–5.19 (m, OH), 5.29 (d, $J = 6.8$ Hz, OH), 5.85 (s, 1H, H-1′), 7.12–7.15 (m, 1H), 7.56 (d, 1H, $J = 2.4$ Hz), 7.62 (d, 1H, $J = 5.2$ Hz), 7.89 (s, 1H), 8.25 (s, 1H), 13.23 (br s, NH). LCMS Calcd for $C_{17}H_{17}N_5O_5S$ 403.4; observed (M + 1) 404.5.

*4.1.1.32. 3-(β-D-2-C-Methyl-ribofuranosyl)-3,9-dihydro-6-(3-thienyl)-9-oxo-5*H*-imidazo-[1,2-*a*]purine (**40**).* ^{1}H NMR (DMSO-d_6) δ 0.79 (s, 3H, CH_3), 3.64–3.68 (m, 1H), 3.78–3.85 (m, 2H), 3.97–4.02 (m, 1H), 5.09 (s, OH), 5.17–5.19 (m, OH), 5.28 (d, $J = 6.8$ Hz, OH), 5.85 (s, 1H, H-1′), 7.67–7.68 (m, 2H), 7.95–7.96 (m, 1H), 7.95 (s, 1H), 8.26 (s, 1H), 13.01 (br s, NH). LCMS Calcd for $C_{17}H_{17}N_5O_5S$ 403.4; observed (M + 1) 404.4.

4.2. LC/MS/MS method for stability studies

The HPLC system was a Dionex Packing Ultimate 3000 modular LC system consisting of a quaternary pump, vacuum degasser, thermostated autosampler, and thermostated column compartment (Dionex, CA). A TSQ Quantum Ultra triple quadrupole mass spectrometer (Thermo Electron Corp.) was used for detection. Thermo Xcalibur software version 1.3 was used to control the HPLC, the mass spectrometer and to perform data analysis. The separation was performed on a Hypersil GOLD-C18 column (50 × 1 mm, 3 μm particle size) (Thermo Scientific, Waltham, MA, USA). The mobile phase A consisted of 10 mM ammonium formate buffer, pH 3 adjusted using formic acid. An alternative mobile phase A consisted of water only. The mobile phase B consisted of methanol. The initial conditions were 95% A and 5% B at 50 μl/min. From 1 min to 2 min B was increased from 5% to 95% and decreased to 5% after 4 min. The total run time was 15 min (including time for column regeneration). The column temperature was kept constant at 30 °C. Analytes were protonated by electrospray ionization (ESI) in positive mode. Selected Reaction Monitoring (SRM) mode was used for the acquisition. The intensity of selected product ion in the MS/MS spectrum of each compound was optimized using direct infusion of the analytes in the corresponding mobile phase (at concentration 100 μM) separately into the instrument using a syringe pump at 5 μl/min. The sheath, ion sweep and auxiliary gas (nitrogen) were set at 50, 0.5 and 1 arbitrary units (au) respectively. The collision gas (argon) pressure was set at 1.5 mTorr. The spray voltage was 4000 V. The capillary was heated at 300 °C. 0.1 s scan time was used for all transitions. The collision-induced dissociation (CID) was used at −6 V. Tube lens was 107 V and collision energy was 20 V. The following *m*/*z* transitions were used for **38** *m*/*z* 441 → 295, **32** *m*/*z* 423 → 282, **33** *m*/*z* 464 → 323, **7**: *m*/*z* 293 → 152, **36** *m*/*z* 428 → *m*/*z* 282, **39** *m*/*z* 404 → 258, **8**: *m*/*z* 298 → 152, **3** 356 → 282 and ACV 226 → 152. 3TC was used as internal standard, with the following SRM: *m*/*z* 230 → 112.

4.2.1. Detection of nucleoside MP, DP and TP intracellularly

4.2.1.1. Cell culture and drug extraction. The 2′-C-methyl analogs were incubated at 50 μM in Huh-7 cells at 37 °C for 4 h. The cells were washed twice with 1 × ice cold PBS and lysed using 60% methanol. After centrifugation to remove cellular debris, the supernatant was evaporated to dryness under a stream of air. Dried extracts were maintained at −80 °C until analysis.

4.2.1.2. Sample preparation. To separate the nucleoside mono-, di- and triphosphates and unchanged nucleosides, half of the samples was reconstituted in 200 μL water containing AZT, AZT-MP and AZT-TP 50 nM and subjected to an anion exchange Solid Phase Extraction. QMA cartridges were conditioned by rinsing with 750 μl of distilled dionized H_2O (ddH_2O). The samples were loaded onto the cartridges followed by a rinse with 300 μl ddH_2O. Unchanged nucleosides were collected first with 750 μl ddH_2O. The cartridge was conditioned with 750 μl of ddH_2O and 300 μl 20 mM KCl. Nucleoside mono-, di- and triphosphates were removed by sequential washes with 750 μl of 100 mM KCl, 120 mM KCl and 400 mM KCl, respectively. The cartridge was rinsed with 150 μl of the corresponding buffer between each. The fractions were collected for de-phosphorylation.

The internal standard (3TC, 10 μl of a solution at 1 μM) was added to the fractions at this point. The pH was lowered to 4.5 by the addition of 400 mM ammonium acetate buffer, and phosphate groups were removed by addition of 2 unit of type XA sweet potato acid phosphatase per ml eluent and incubation at 37 °C for 1 h. To remove salt from the resulting fractions, the samples were loaded onto Waters OASIS HLB cartridges pre-conditioned with 2 ml MeOH and 2 ml ddH_2O, and washed with 3 ml of ddH_2O. Nucleoside analogs of interest were eluted with 1 ml of methanol and dried under a stream of air. The residue was reconstituted with 100 μl. The other half of the samples was directly reconstituted with 100 μl of water containing 100 nM 3TC which were filtered by ultrafiltration (COSTAR filters 0.2 μm). Fractions from both SPE and direct reconstitution were injected onto the LC–MS/MS system for intracellular nucleoside detection. The method described previously (Section 4.2) was used.

4.2.2. Conditions for stability studies

A known concentration of the analogs was diluted in two separate fractions, water and methanol, directly from a stock solution synthesized the same day and injected onto the LC/MS/MS using the method previously described in Section 4.2. Injections were performed every 15 min for the first two hours, then every hour, every day and finally every week. Half of the sample was kept at room temperature and the other half was kept in the autosampler at 4 °C. Standard calibration of the nucleosides **7**, **8** and ACV ranging from 10 to 1000 nM were used in order to calculate the percentage of G analog formation.

4.3. Antiviral activity evaluation

The antiviral activity of compounds **9**–**40** was evaluated against HIV [27] in activated primary human PBM cells and in Huh-7 cells with a HCV subgenomic RNA replicon system [28]. Cytotoxicity was evaluated in the HCV replicon and normal PBM cells, along with CEM and Vero cells [29]. Compound **3** was evaluated for activity against HSV-1 (strain F) by plaque reduction assay in Vero cells using methodologies described previously [30].

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