



Research paper

Modification of the length and structure of the linker of N^6 -benzyladenosine modulates its selective antiviral activity against enterovirus 71



Mikhail S. Drenichev^{a,1}, Vladimir E. Oslovsky^{a,1}, Liang Sun^b, Aloys Tijjsma^b, Nikolay N. Kurochkin^a, Vitali I. Tararov^a, Alexander O. Chizhov^c, Johan Neyts^b, Christophe Pannecouque^b, Pieter Leyssen^b, Sergey N. Mikhailov^{a,*}

^a Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Vavilov Str. 32, Moscow 119991, Russian Federation

^b KU Leuven – University of Leuven, Department of Microbiology and Immunology, Laboratory for Virology and Chemotherapy, Rega Institute for Medical Research, Minderbroedersstraat 10, 3000 Leuven, Belgium

^c Zelinsky Institute of Organic Chemistry, Russian Academy of Science, Leninsky pr., 47, Moscow 119991, Russian Federation

ARTICLE INFO

Article history:

Received 25 November 2015

Received in revised form

18 January 2016

Accepted 19 January 2016

Available online 26 January 2016

Keywords:

N^6 -benzyladenosine derivatives

Synthesis and antiviral activity

SAR

Enterovirus 71

ABSTRACT

Very recently, we demonstrated that N^6 -isopentenyladenosine, a cytokinin nucleoside, exerts a potent and selective antiviral effect on the replication of human enterovirus 71. The present study is devoted to the structure optimization of another natural compound: N^6 -benzyladenosine. We mainly focused on the exploration of the size and nature of the linker between the adenine and the phenyl ring, as well as on the necessity of the D-ribose residue. More than 30 analogues of N^6 -benzyladenosine were prepared and their antiviral properties were evaluated. Two main methodologies were used for preparation: N^6 -acetyl-2',3',5'-tri-*O*-acetyladenosine can be regioselectively alkylated either by alkyl halides under base promoted conditions or by alcohols in Mitsunobu reactions. After deacylation with 4 M PrNH₂ in MeOH at room temperature for one day, the desired products were obtained in overall high yields. Analysis of the structure-activity relationship clearly shows that the optimal size of the linker is limited to 2 or 3 atoms (compounds 4–7). 2'-Deoxyadenosine derivatives did not elicit any inhibitory or cytotoxic effect, while 5'-deoxynucleosides still induced some cell protective antiviral activity. Based on these observations, it can be hypothesized that there may be another mechanism that is at the base of the antiviral activity of these compounds against enterovirus 71 besides a possible 5'-triphosphorylation followed by a putative inhibitory effect on RNA synthesis.

© 2016 Elsevier Masson SAS. All rights reserved.

1. Introduction

Enterovirus 71 (EV71) is a non-enveloped, single-stranded, positive-sense RNA virus that belongs to the *Picornaviridae* family. The virus is, together with Coxsackievirus A16 (CV-A16), a major causative agent of hand-, foot-, and mouth disease (HFMD), a mild and self-limiting illness that mostly affects children under the age of 5. However, in some patients, the virus can cause severe, potentially lethal complications such as aseptic meningitis, encephalitis, pulmonary edema, and viral myocarditis [1, 2]. In recent

years, EV71 has caused large outbreaks of HFMD in Asia, during which an increased number of severe neurological complications, such as encephalitis and acute flaccid paralysis, have been observed [3]. Furthermore, in the past ten years, EV71 infections have also been reported in countries in America and Europe [4].

Even though several promising EV71 vaccine candidates are currently in clinical trial [5], to date, there is still no approved vaccine available for prophylaxis [6–8]. In addition, it may also be important to have effective antivirals at hand for the treatment of infected patients with severe disease [9–11]. At the moment there are still no antivirals on the market for the treatment of enterovirus infection.

In 2008, Arita and coworkers identified 4 inhibitors of EV71 replication during a screen of the LOPAC¹²⁸⁰ drug library (Sigma-Aldrich) [12]. Two of these compounds were nucleoside analogs, i.e.

* Corresponding author.

E-mail address: smikh@eimb.ru (S.N. Mikhailov).

¹ Both authors contributed equally.

N^6 -benzyladenosine (**23**) and N^6 -(*o*-methylbenzyl)adenosine (metrifudil). In 2014, Shang and colleagues reported on the anti-EV71 activity of several natural and synthetic nucleosides. In particular N^6 -benzyladenosine and 2'-ethynyladenosine were shown to have significant antiviral activity against EV71 with an EC_{50} of 5 μ M and 0.625 μ M, respectively [13]. Recently, our research groups demonstrated that N^6 -isopentenyladenine, a natural plant cytokinin (**31**), also inhibits the replication of EV71 with an EC_{50} of 1.0 ± 0.2 μ M [14]. Natural products have been the major sources of drugs in medicinal chemistry. An approach which is based on the structure of natural compounds is rather popular and fruitful [15–17]. For example, about 100 nucleoside-based drugs have been developed and nucleoside library is a promising pool for the fishing of new biologically active compounds [18, 19].

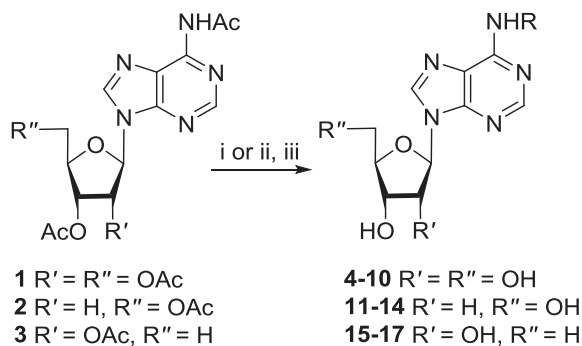
In the present study, we report on the exploration of the properties of the linker structure and length of N^6 -benzyladenosine-based compounds in an attempt to obtain more potent and selective inhibitors of the replication of EV71.

2. Results and discussion

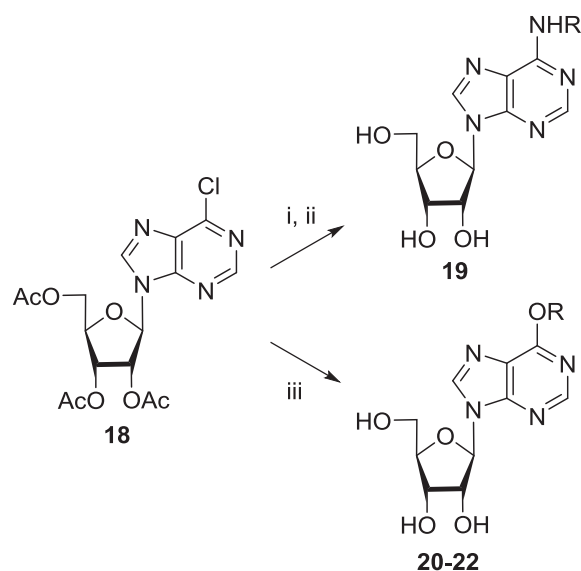
2.1. Chemistry

Recently, we have developed a new useful and versatile approach for the preparation of N^6 -adenosine derivatives by regioselective N^6 -alkylation of N^6 -acetyl-2',3',5'-tri-*O*-acetyladenosine (**1**) [20] with alcohols under Mitsunobu conditions or with alkyl halides promoted by a base (Scheme 1). The traditional approach for the preparation of N^6 -alkylated adenosines is the substitution of the chlorine atom in commercially available 6-chloropurine riboside with alkylamines [21, 22] (Scheme 2). The main advantage of our method is the possibility to use both alkyl halides and alcohols for N^6 -modification. This is important, especially in the case when an amine is not stable or hardly available. The only difficulty was associated with purification of the final nucleosides from the by-products caused by the Mitsunobu reaction and acetamide forming under ammonolysis conditions. We concluded that, for this class of compounds, the method of choice was deacetylation with 4 M $PrNH_2$ in MeOH, which significantly facilitated chromatographic purification [14].

The developed protocol for N^6 -alkylation was used successfully for the preparation of isomeric deoxyadenosine derivatives starting from triacetyl-2'-deoxyadenosine **2** and triacetyl-5'-deoxyadenosine **3**. The key intermediate **3** was prepared by radical Bu_3SnH reduction of corresponding 5'-chloro-5'-deoxyadenosine derivative in a way similar to 5'-deoxyuridine synthesis [23].



Scheme 1. Synthesis of N^6 -alkyladenosines, N^6 -alkyl-2'-deoxyadenosines and N^6 -alkyl-5'-deoxyadenosines. Reagents and conditions: (i) ROH, Ph_3P , DEAD, THF, 20 °C, 24–48 h; (ii) RBr, DBU, CH_3CN , r.t., 1–3 days; (iii) 4 M $PrNH_2$ in MeOH, 20 °C, 24 h (The structure of R is given in Tables 1, 3, 4).



Scheme 2. Synthesis of N^6 -phenyladenosine and O^6 -substituted inosine derivatives by substitution of chlorine atom in 2',3',5'-tri-*O*-acetyl-6-chloropurineriboside (**18**). Reagents and conditions: (i) RNH_2 , *n*-BuOH, DIPEA, 80 °C; (ii) 7 M $NH_3/MeOH$, r.t., 24 h; (iii) DMSO, *t*-BuOK, ROH, 20 °C, 24 h (The structure of R is given in Tables 1–4).

The preparation of a series of N^6 -aryladenosines and O^6 -alkyladenosines (Scheme 2) was started with 2',3',5'-tri-*O*-acetyl-6-chloropurineriboside (**18**). The substitution of chloride with various nucleophiles in unprotected 6-chloropurineriboside is well-known [21, 22]. 6-Chloropurineriboside can be prepared by deacetylation of **18** [24]. To simplify the procedure, we used compound **18** directly in the substitution reactions. Interestingly, acetyl groups are completely preserved in the reaction with aniline and the protected intermediate can be isolated and characterized. The removal of acetyl groups by ammonolysis affords nucleoside **19**. This is quite opposite to the reactions with alkylamines and alcohols, in which the complete deacetylation has been observed. The isolation of inosine derivatives **20–22** was performed by column chromatography (silica gel) of the whole reaction mixtures after neutralization with neat acetic acid.

The structure of compounds was confirmed by NMR spectral data. The distinguishing feature of unprotected adenosines and their parent bases is the significant broadening of signals of the CH_2 group attached to the N^6 -atom. Such effect is general for N^6 -substituted nucleosides and their bases. The possible explanation can be found in the literature and is ascribed to 'a chemical exchange process connected to the restricted conformational change' [25, 26]. Noteworthy, broadening is not observed for the same compounds when a protective N^6 -acyl group is present and for corresponding O^6 -inosine derivatives.

2.2. Biological activity

N^6 -Substituted adenines (cytokinins) represent an important group of phytohormones that are involved in numerous biochemical processes in plants, stimulating cell division and regulating plant growth. In nature, cytokinins can be found with an aromatic side chain (N^6 -benzyladenine and its hydroxylated derivatives—topolins), with an isoprenoid side chain, such as N^6 -(Δ^2 -isopentenyl)adenine (iP), and as their hydroxylated derivatives—zeatins. The biosynthesis is only well understood in the case of iP. Cytokinins can occur in nature in the form of free bases, nucleosides (*N*-glycosides) and nucleotides. Overall, it is expected that the plant cytokinin diversity could be constituted out of up to one

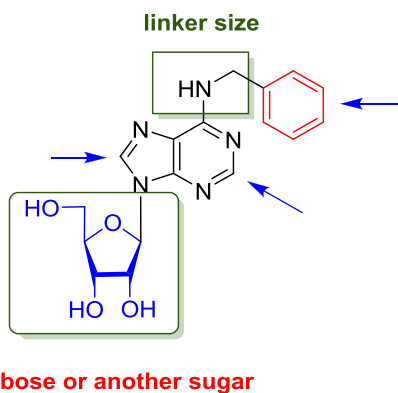


Fig. 1. Possible modifications of N^6 -benzyladenosine (**23**).

hundred derivatives. Significant and different activities have been found only in the case of cytokinin nucleosides, which manifest antitumor, antiviral, antiprotozoal, cytokinin, blood pressure reducing, anti-inflammatory, antipsychotic activity, influence platelet aggregation and some others [14, 27–30]. One of the best studied derivatives of this group is N^6 -benzyladenosine **23**, which was synthesized in the late 60ies [21], and which only quite recently was found and characterized in plants [31].

Taking into consideration the earlier findings [12] and our recent observations [14], we selected N^6 -benzyladenosine (**23**) as a promising compound with anti-enterovirus activity for further optimization. The structure of **23** has a lot of different functionalities and may be modified in several ways as marked with the arrows on Fig. 1. In the present investigation, we have mainly focused on the size and nature of the linker between the purine nucleus and

Table 1
Antiviral activity of adenosine derivatives on the replication of EV71 strain BrCr in RD cells.

Compound name	Substituent (R)	CC ₅₀ ± SD	EC ₅₀ ± SD ^{a,b}	SI ^c
19 N^6 -phenyladenosine		372 ± 16	>291	ND
23 N^6 -benzyladenosine		4 ± 1	0.28 ± 0.05	14
4 N^6 -(2-phenylethyl)-adenosine		221 ± 32	1.8 ± 0.6	123
5 N^6 -(3-phenylpropan-1-yl)-adenosine		189 ± 27	1.8 ± 0.6	105
6 N^6 -(trans-3-phenyl-2-propen-1-yl)-adenosine		426 ± 7	0.66 ± 0.12	645
7 N^6 -(3-phenyl-2-propin-1-yl)-adenosine		72 ± 4	0.58 ± 0.13	124
8 N^6 -(2-phenoxyethyl)-adenosine		107 ± 40	4.3 ± 0.2	25
24 N^6 -(Benzyloxymethyl)adenosine		525 ± 36	3.0 ± 0.3	175
9 N^6 -(4-phenylbutane-1-yl)-adenosine		>313	>250	ND
10 N^6 -(3-phenoxypropan-1-yl)-adenosine		>311	>311	ND
25 N^6 -benzoyladenosine		>337	12 ± 2	>28
26 N^6 -furfuryladenosine		7.8 ± 3.4	1.4 ± 0.3	5.6
27 N^6 -(1-Methyl-1,2,3-triazol-4-yl-methyl)adenosine		136 ± 8	>136	ND
28 N^6 -[1-(2-hydroxyethyl)-1,2,3-triazol-4-yl-methyl]adenosine		317 ± 30	>317	ND
29 N^6 -(1-benzyl-1,2,3-triazol-4-yl-methyl)adenosine		>285	>228	ND
30 N^6 -(1-benzyloxymethyl-1,2,3-triazol-4-yl-methyl)adenosine		116 ± 29	>116	ND
31 N^6 -isopentenyladenosine		5.8 ± 0.5	1.0 ± 0.2	5.8
32 N^6 -(5-hexene-2-yne-1-yl)adenosine		>434	4.9 ± 1.1	>88

ND = Not Determined.

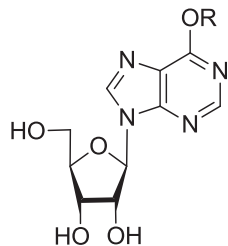
^a All values are in μ M and are based on at least three independent dose-response curves.

^b On rhabdomyosarcoma (RD) cells.

^c Selectivity Index (SI); SI = CC₅₀/EC₅₀.

Table 2

Antiviral effect of inosine derivatives on the replication of EV71 strain BrCr in RD cells.

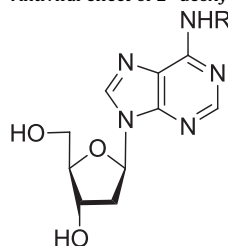


	Compound name	Substituent (R)	CC ₅₀ ± SD	EC ₅₀ ± SD ^{a,b}	SI ^c
20	O ⁶ -benzylinosine		191 ± 39	5.1 ± 1.9	37
21	O ⁶ -(2-phenylethyl)-inosine		8.5 ± 4.1	>8.5	ND
22	O ⁶ -(3-phenylpropan-1-yl)-inosine		182 ± 46	>182	ND

ND = Not Determined.

^a All values are in μM and are based on at least three independent dose-response curves.^b On rhabdomyosarcoma (RD) cells.^c Selectivity Index (SI); SI = CC₅₀/EC₅₀.**Table 3**

Antiviral effect of 2'-deoxyadenosine derivatives on the replication of EV71 strain BrCr in RD cells.

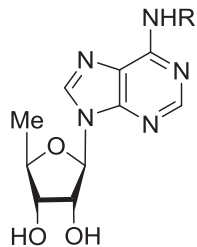


	Compound name	Substituent (R)	CC ₅₀ ± SD	EC ₅₀ ± SD ^{a,b}	SI ^c
11	N ⁶ -benzyl-2'-deoxyadenosine		>366	>146	ND
12	N ⁶ -(2-phenylethyl)-2'-deoxyadenosine		>352	>281	ND
13	N ⁶ -(3-phenylpropan-1-yl)-2'-deoxyadenosine		>271	>271	ND
14	N ⁶ -(2-phenoxyethyl)-2'-deoxyadenosine		>337	>135	ND

ND = Not Determined.

^a All values are in μM and are based on at least three independent dose-response curves.^b On rhabdomyosarcoma (RD) cells.^c Selectivity Index (SI); SI = CC₅₀/EC₅₀.**Table 4**

Antiviral effect of 5'-deoxyadenosine derivatives on the replication of EV71 strain BrCr in RD cells.

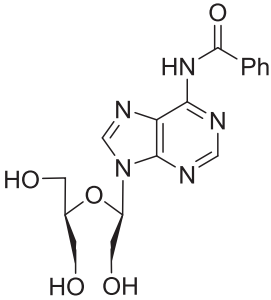


	Compound name	Substituent (R)	CC ₅₀ ± SD	EC ₅₀ ± SD ^{a,b}	SI ^c
15	N ⁶ -isopentenyl-5'-deoxyadenosine		182 ± 79	13 ± 2	14
16	N ⁶ -benzyl-5'-deoxyadenosine		>366	6.7 ± 1.6	>54
17	N ⁶ -(2-phenylethyl)-5'-deoxyadenosine		463 ± 29	>281	ND

ND = Not Determined.

^a All values are in μM and are based on at least three independent dose-response curves.^b On rhabdomyosarcoma (RD) cells.^c Selectivity Index (SI); SI = CC₅₀/EC₅₀.

Table 5
Antiviral effect of acyclic adenosine derivative on the replication of EV71 strain BrCr in RD cells.

Compound name	Structure	CC ₅₀ ± SD	EC ₅₀ ± SD ^{a,b}	SI ^c
33 N-(9-(1-[(1,3-dihydroxypropan-2-yl)oxy]-2-hydroxyethyl)-9H-purin-6-yl)benzamide		>335	>335	ND

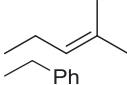
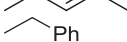
ND = Not Determined.

^a All values are in μM and are based on at least three independent dose-response curves.

^b On rhabdomyosarcoma (RD) cells.

^c Selectivity Index (SI); $\text{SI} = \text{CC}_{50}/\text{EC}_{50}$.

Table 6
Antiviral effect of cytokinins on the replication of EV71 strain BrCr in RD cells.

Compound name	Substituent (R)	CC ₅₀ ± SD	EC ₅₀ ± SD ^{a,b}	SI ^c
34 N ⁶ -isopentenyladenine		325 ± 18	>325	ND
35 N ⁶ -benzyladenine		547 ± 14	0.42 ± 0.05	1302

ND = Not Determined.

^a All values are in μM and are based on at least three independent dose-response curves.

^b On rhabdomyosarcoma (RD) cells.

^c Selectivity Index (SI); $\text{SI} = \text{CC}_{50}/\text{EC}_{50}$.

Table 7
Antiviral effect of compounds **4** and **6** against a selection of EV71 clinical isolates.

Genogroup	Strain	Genbank	EC ₅₀ ± SD ^a	
			4	6
B2	11316	AB575927	8.2 ± 2.0	2.2 ± 0.1
B5	TW/96016/08	GQ231942	5.6 ± 1.1	2.0 ± 0.3
	TW/70902/08	GQ231936	8.3 ± 1.0	2.5 ± 0.4
C2	H08300 461#812	–	1.9 ± 0.2	0.38 ± 0.03
C4	TW/1956/05	GQ231926	2.7 ± 1.0	1.0 ± 0.4
	TW/2429/04	GQ231927	3.7 ± 0.2	1.16 ± 0.01

^a All values are in μM and are based on at least three independent dose-response curves.

the phenyl ring, as well as on the necessity of the D-ribose residue. The evaluation of the antiviral activity of the compounds in an enterovirus EV71 cell-based assay revealed that the selectivity index can be significantly improved by variation of the linker structure.

N⁶-Benzyladenosine **23** was found to be rather cytotoxic (Table 1), so, one angle to obtain compounds with an improved selectivity index was to try to reduce the cytotoxic effect.

We have previously shown that a hydrophobic residue is essential for antiviral activity against enterovirus 71 [14]. Therefore, we prepared different N⁶-benzyladenosine analogs with different properties of the linker between the purine and the phenyl group, changing from zero atoms (**19**) up to four (**9** and **10**) and with

different character, containing not only polymethylene and oxamethylene chain, but also double and triple bonds. Our SAR study clearly shows that the optimal size of the linker appears to be limited to 2 or 3 atoms (compounds **4**–**7**). Compounds **8** and **24**, having a more polar oxamethylene linker, were less active. N⁶-Phenyladenosine (**19**), without linker, and **9** and **10** (4-atoms linker) were practically inactive.

Other cytokinin ribosides, **31** and **26**, were less active than N⁶-benzyladenosine **23**. It is quite interesting to observe that N⁶-benzoyladenosine (**25**) possesses anti-enterovirus 71 activity without a pronounced adverse effect on the host cells. Compounds with triazole heterocycles (**27**–**30**) were practically inactive.

Inosine derivatives **20**–**21** (Table 2) were found to be much less effective than the corresponding adenosines. Their antiviral activity decreases with an increase of the linker length.

The modulation of the length and nature of the linker between purine and phenyl group can definitely improve the selectivity of the start compound **23** (Supporting info, Figs. 1–3).

2'-Deoxyadenosine derivatives **11**–**14** (Table 3) do not have any apparent antiviral activity or cytotoxicity. In the 5'-deoxynucleoside series **15**–**16** (Table 4), the antiviral activity was substantially decreased compared to the natural cytokinin nucleosides **23** and **31**.

The acyclic derivative **33** (Table 5) without intact ribose was not active compared to the related N⁶-benzoyladenosine (**25**).

In the previous paper [14], we have shown that natural N⁶-isopentenyladenine (**34**) (Table 6) does not protect the cells from virus-induced cell death. In contrast to this observation, N⁶-benzyladenine (**35**) has antiviral activity comparable to that of the start nucleoside **23**.

Biological activity of cytokinin nucleosides may be associated not only with the compounds as described in this study, but also with phosphorylated metabolites that may become available following intracellular metabolism of the parent compound. It was shown earlier that cytokinins may be converted to their corresponding nucleoside 5'-monophosphates [32].

It was also shown [33] that 6-furyl- or 6-thienyl-7-deazapurines ribosides induced very high cytotoxicity on several tumor cell lines and that they required 5'-triphosphorylation to be able to act by inhibition of RNA synthesis.

Our data are in line with these observations: the antiviral activity is absent for 2'-deoxyadenosine derivatives **11**–**14** and the acyclic nucleoside **33**. 5'-Deoxynucleosides **15**–**16**, which do not have 5'-hydroxyl group and could not be converted to the 5'-monophosphates, have much lower antiviral activity than the

related ribonucleosides. But the remaining activity in **15**–**16** points out that 5'-triphosphorylation and inhibition of RNA synthesis cannot be the only mechanism of antiviral activity of cytokinin nucleosides. The antiviral activity of N^6 -benzyladenine **35** is in line with this conclusion.

Finally, the antiviral activity of compound **4** and **6**, being the compounds with the highest selectivity index of the entire series, was evaluated on the replication of a representative panel of clinical isolates of EV71 (Table 7). The replication of the virus isolates that belong to genogroups B2 and B5 appears to be 2-fold less sensitive to the antiviral effect of both compounds compared to that of genogroup C4. In turn, both compounds more potently inhibited the replication of the virus isolate that belongs to genogroup C2. Along the line, compound **6** consistently shows up as the most potent and selective (highest selectivity index) inhibitor of this series.

3. Conclusions

We demonstrated that the antiviral activity of natural N^6 -benzyladenosine derivatives is greatly dependent on the size of the linker and that a linker with a length of 2–3 atoms has the most pronounced antiviral activity. Furthermore, the nature of the linker is also of great importance, as compounds with double and triple bonds have better activity than those with polymethylene and oxamethylene chains. As a result of the optimization, the selectivity index could be improved with a factor of 50 (transition of **23** to **6**), an increase that is mainly to be attributed to a reduction in the adverse effect of this compound on the host cells (increased CC₅₀).

4. Experimental section

4.1. General

The solvents and materials were reagent grade and were used without additional purification. Column chromatography was performed on silica gel (Kieselgel 60 Merck, 0.063–0.200 mm). TLC was performed on Alugram SIL G/UV254 (Macherey-Nagel) with UV visualization. Melting points were determined with Electro-thermal apparatus and are uncorrected. ¹H and ¹³C (with complete proton decoupling) NMR spectra were recorded on Bruker AMX 400 NMR instrument at 300 K. ¹H-NMR-spectra were recorded at 400 MHz and ¹³C-NMR-spectra at 100 MHz. Chemical shifts in ppm were measured relative to the residual solvent signals as internal standards (CDCl₃, ¹H: 7.26 ppm, ¹³C: 77.1 ppm; DMSO-*d*₆, ¹H: 2.50 ppm, ¹³C: 39.5 ppm [34]. Spin-spin coupling constants (*J*) are given in Hz. Double resonance technique was applied for assigning the resonances. High resolution mass spectra (HRMS) were measured on a Bruker micrOTOF II instrument using electrospray ionization (ESI) [35]. The measurements were done in a positive ion mode (interface capillary voltage – 4500 V) or in a negative ion mode (3200 V); mass range from *m/z* 50 to *m/z* 3000 Da; external or internal calibration was done with Electrospray Calibrant Solution (Fluka). A syringe injection was used for solutions in acetonitrile, methanol, or water (flow rate 3 μL/min). Nitrogen was applied as a dry gas; interface temperature was set at 180 °C.

The following compounds were prepared according to the methods reported elsewhere:

N^6 -Acetyl-2',3',5'-tri-*O*-acetyladenosine (**1**), N^6 -benzyladenosine (**23**), N^6 -furfuryladenosine (**26**) were prepared according to ref. [20]; N^6 -(benzyloxymethyl)adenosine (**24**), O^6 -benzylinosine (**20**), N^6 -(1-Methyl-1,2,3-triazol-4-yl-methyl)adenosine (**27**), N^6 -[1-(2-hydroxyethyl)-1,2,3-triazol-4-yl-methyl]adenosine (**28**), N^6 -(1-benzyl-1,2,3-triazol-4-yl-methyl)adenosine (**29**), N^6 -(1-benzyloxymethyl-1,2,3-triazol-4-yl-methyl)adenosine (**30**) were prepared according to ref. [27]; N^6 -benzoyladenosine (**25**) was

prepared according to ref. [36]; *N*-(9-(1-[(1,3-dihydroxypropan-2-yl)oxy]-2-hydroxyethyl)-9*H*-purin-6-yl)benzamide (**33**) was prepared according to ref. [37].

4.2. Adenosine derivatives

4.2.1. N^6 -Phenyl-2',3',5'-tri-*O*-acetyl-adenosine

A mixture of **18** [24, 38] (193 mg, 0.467 mmol) and aniline hydrochloride (70 mg, 0.540 mmol) was solved in *n*-BuOH (5 ml) and then DIPEA (0.185 ml, 1.08 mmol) was added in one portion. The solution was stirred at 80 °C. The reaction was monitored by TLC (ethyl acetate:hexane – 2:1). After 14 h, the reaction mixture was evaporated in vacuum and the residue was diluted with methylene chloride (30 ml) and washed with water (2 × 15 ml). The organic layer was separated, dried over anhydrous Na₂SO₄ and evaporated in vacuum. The residue was purified by column chromatography on silica gel. The product was eluted with CH₂Cl₂–EtOH (97:3) mixture. Yield of N^6 -phenyl-2',3',5'-tri-*O*-acetyl-adenosine was 219 mg (93%) as foam. *R*_f 0.42 (ethyl acetate-hexane, 2:1 v/v). ¹H NMR (400 MHz, CDCl₃): δ = 2.09 (s, 3H, Ac), 2.13 (s, 3H, Ac), 2.15 (s, 3H, Ac), 4.37–4.49 (m, 3H, H4', H5'a, H5'b), 5.64 (dd, 1H, *J*_{3',2'} = 5.3 Hz, *J*_{3',4'} = 4.5 Hz, H3'), 5.91 (dd, 1H, *J*_{2',1'} = 5.2 Hz, *J*_{2',3'} = 5.3 Hz, H2'), 6.22 (d, 1H, *J*_{1',2'} = 5.2 Hz, H1'), 7.15–7.22 (m, 1H, *H*_p-Ph), 7.37–7.44 (m, 2H, *H*_m-Ph), 7.72–7.78 (m, 2H, *H*_o-Ph), 8.05 (s, 1H, H8), 8.51 (s, 1H, H2).

4.2.2. N^6 -Phenyladenosine (**19**)

N^6 -phenyl-2',3',5'-tri-*O*-acetyl-adenosine (116 mg, 0.247 mmol) was treated with 7 M NH₃ in MeOH solution (3 ml). After 24 h, the mixture was evaporated in vacuum. The product was recrystallized from MeOH. The precipitant was filtered, washed with cold MeOH and dried over P₂O₅. Yield of **19** was 69 mg (81%) as a white powder. *R*_f 0.22 (CH₂Cl₂–EtOH, 10:1 v/v). mp 199–200 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 3.58 (ddd, 1H, *J*_{5'b,4'} = 3.5 Hz, *J*_{5'b,OH} = 6.7 Hz, *J*_{5'b,5'a} = –12.1 Hz, H5'b), 3.70 (ddd, 1H, *J*_{5'a,4'} = 3.5 Hz, *J*_{5'a,OH} = 4.8 Hz, *J*_{5'a,5'b} = –12.1 Hz, H5'a), 3.98 (ddd, 1H, *J*_{4',3'} = 3.5 Hz, *J*_{4',5'a} = 3.5 Hz, *J*_{4',5'b} = 3.5 Hz, H4'), 4.19 (dd, 1H, *J*_{3',2'} = 4.8 Hz, *J*_{3',4'} = 3.5 Hz, H3'), 4.65 (dd, 1H, *J*_{2',1'} = 6.0 Hz, *J*_{2',3'} = 4.8 Hz, H2'), 5.17 (d, 1H, *J*_{3',OH} = 4.8 Hz, 3'OH), 5.25 (dd, 1H, *J*_{5'a',OH} = 4.8 Hz, *J*_{5'b',OH} = 6.7 Hz, 5'OH), 5.45 (d, 1H, *J*_{2',OH} = 6.2 Hz, 2'OH), 5.97 (d, 1H, *J*_{1',2'} = 6.0 Hz, H1'), 7.02–7.08 (m, 1H, *H*_p-Ph), 7.30–7.36 (m, 2H, *H*_m-Ph), 7.91–7.97 (m, 2H, *H*_o-Ph), 8.40 (s, 1H, H8), 8.53 (s, 1H, H2), 9.89 (s, 1H, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ = 61.56 (C5'), 70.56 (C3'), 73.60 (C2'), 85.86 (C4'), 87.86 (C1'), 120.36 (C5), 120.90 (Ph), 122.74 (Ph), 128.40 (Ph), 139.52 (Ph), 140.71 (C8), 149.33 (C4), 151.93 (C2), 152.17 (C6). HRMS: *m/z* [M+Na]⁺ calculated C₁₆H₁₇N₅O₄Na⁺ 366.1173, found 366.1167.

4.2.3. N^6 -(2-Phenylethyl)-adenosine (**4**)

To a solution of **1** (435 mg, 1 mmol), Ph₃P (393 mg, 1.5 mmol) and 2-phenylethanol (0.180 ml, 1.5 mmol) in THF (5 ml) DEAD (0.24 ml, 1.5 mmol) was added in one portion and the solution was kept at ambient temperature for 20 h. The reaction was monitored by TLC (silica gel, CH₂Cl₂–EtOH, 97:3). Then, the reaction mixture was evaporated and the residue was applied to column chromatography (silica gel, CH₂Cl₂–EtOH = 97:3). Partially purified compound was dissolved in 4 M PrNH₂ in MeOH solution (50 mmol) and was left for 24 h, after which the mixture was evaporated and the residue was applied to column chromatography. The column was washed with methylene chloride:ethanol – 95:5 and then eluted with methylene chloride:ethanol – 90:10 to give **4** as a white powder. Yield 180 mg (65%). *R*_f 0.28 (CH₂Cl₂–EtOH, 9:1). mp 169–171 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 2.93 (t, 2H, *J*_{CH₂-CH₂} = 7.5 Hz, *C*_{H₂}Ph), 3.55 (ddd, 1H, *J*_{5'b,5'a} = –12.0 Hz, *J*_{5'b,4'} = 3.4 Hz, *J*_{5'b,OH} = 7.0 Hz, H5'b), 3.63–3.81 (m, 3H, NHCH₂,

H5'a), 3.97 (ddd, 1H, $J_{4',5'b} = 3.0$, $J_{4',5'a} = 3.4$, $J_{4',3'} = 3.2$, H4'), 4.15 (ddd, 1H, $J_{3',4'} = 3.2$ Hz, $J_{3',2'} = 5.9$ Hz, $J_{3',OH} = 4.6$ Hz, H3'), 4.60 (dd, 1H, $J_{2',3'} = 5.9$ Hz, $J_{2',1'} = 6.1$ Hz, H2'), 5.15 (d, 1H, $J_{OH,3'} = 4.6$ Hz, 3'OH), 5.36 (dd, 1H, $J_{OH,5'b} = 7.0$ Hz, $J_{OH,5'a} = 4.6$ Hz, 5'OH), 5.41 (d, 1H, $J_{OH,2'} = 6.1$ Hz, 2'OH), 5.89 (d, 1H, $J_{1',2'} = 6.1$ Hz, H1'), 7.16–7.33 (m, 5H, Ph), 7.88 (br s, 1H, NH), 8.23 (br s, 1H, H2), 8.34 (s, 1H, H8). ^{13}C NMR (100 MHz, DMSO- d_6): $\delta = 34.39$ (CH₂Ph), 41.23 (NCH₂), 61.66 (C5'), 70.63 (C3'), 73.49 (C2'), 85.88 (C4'), 87.94 (C1') 119.74 (C5), 126.00 (Ph), 128.27 (Ph), 128.63 (Ph), 139.48 (Ph), 139.70 (C8), 148.27 (C4), 152.35 (C2), 154.61 (C6). HRMS: m/z [M+H]⁺ calculated C₁₈H₂₂N₅O₄⁺ 372.1666, found 372.1657; m/z [M+Na]⁺ calculated C₁₈H₂₁N₅O₄Na⁺ 394.1486, found 394.1474.

4.2.4. N⁶-(3-Phenylpropan-1-yl)-adenosine (5)

Following the procedure for preparation of **4**, condensation of **1** (300 mg, 0.68 mmol) with 3-phenylpropan-1-ol (0.139 ml, 1.02 mmol) in the presence of Ph₃P (269 mg, 1.02 mmol) and DEAD (0.16 ml, 1.02 mmol) in THF (5 ml) with subsequent deblocking in 4 M PrNH₂ in MeOH solution (25 mmol) gave **5** as a white crystals. Yield 208 mg (79%). R_f 0.11 (CH₂Cl₂–EtOH, 97:3), mp 126–129 °C. ^1H NMR (400 MHz, DMSO- d_6): $\delta = 1.91$ (p, 2H, $J_{\text{CH}_2\text{CH}_2} = 7.5$ Hz, CH₂CH₂CH₂Ph), 2.65 (t, 2H, $J_{\text{CH}_2\text{CH}_2} = 7.5$ Hz, CH₂Ph), 3.46–3.60 (m, 3H, H5'b, NHCH₂), 3.67 (ddd, 1H, $J_{5'a,4'} = 3.4$ Hz, $J_{5'a,OH} = 4.0$ Hz, $J_{5'a,5'b} = -12.1$ Hz, H5'a), 3.96 (ddd, 1H, $J_{4',3'} = 3.4$ Hz, $J_{4',5'a} = 3.4$ Hz, $J_{4',5'b} = 3.4$ Hz, H4'), 4.15 (ddd, 1H, $J_{3',2'} = 4.8$ Hz, $J_{3',4'} = 3.4$ Hz, $J_{3',OH} = 4.6$ Hz, H3'), 4.61 (dd, 1H, $J_{2',1'} = 6.1$ Hz, $J_{2',3'} = 4.8$ Hz, H2'), 5.14 (d, 1H, $J_{3',OH} = 4.6$ Hz, 3'OH), 5.35–5.42 (m, 2H, 2'OH, 5'OH), 5.88 (d, 1H, $J_{1',2'} = 6.1$ Hz, H1'), 7.13–7.31 (m, 5H, Ph), 7.91 (br s, 1H, NH), 8.20 (s, 1H, H8), 8.34 (s, 1H, H2). ^{13}C NMR (100 MHz, DMSO- d_6): $\delta = 30.77$ (CH₂), 32.60 (CH₂, CH₂), 61.67 (C5'), 70.64 (C3'), 73.47 (C2'), 83.89 (C4'), 87.96 (C1'), 119.72 (C5), 125.64 (Ph), 128.22 (Ph), 139.60 (C8), 141.78 (Ph), 148.38 (C4), 152.31 (C2), 154.72 (C6). HRMS: m/z [M+H]⁺ calculated C₁₉H₂₄N₅O₄⁺ 386.1823, found 386.1814; m/z [M+Na]⁺ calculated C₁₉H₂₃N₅O₄Na⁺ 408.1642, found 408.1634.

4.2.5. N⁶-(Trans-3-phenyl-2-propen-1-yl)-adenosine (6)

Following the procedure for preparation of **4**, condensation of **1** (435 mg, 1 mmol) with *trans*-3-phenyl-2-propen-1-ol (0.193 ml, 1.5 mmol) in the presence of Ph₃P (393 mg, 1.5 mmol) and DEAD (0.24 ml, 1.5 mmol) in THF (5 ml) with subsequent deblocking in 4 M PrNH₂ in MeOH solution (50 mmol) gave **6** as a white powder. Yield 238 mg (62%). R_f 0.08 (CH₂Cl₂–EtOH, 97:3), mp 129–132 °C. ^1H NMR (400 MHz, DMSO- d_6): $\delta = 3.56$ (ddd, 1H, $J_{5'b,4'} = 3.4$ Hz, $J_{5'b,5'a} = -12.1$ Hz, $J_{5'b,OH} = 6.9$ Hz, H5'b), 3.68 (ddd, 1H, $J_{5'a,4'} = 3.4$ Hz, $J_{5'a,5'b} = -12.1$ Hz, $J_{5'a,OH} = 4.7$ Hz, H5'a), 3.98 (ddd, 1H, $J_{4',3'} = 6.9$ Hz, $J_{4',5'a} = 3.4$ Hz, $J_{4',5'b} = 3.4$ Hz, H4'), 4.20 (dd, 2H, $J_{\text{CH}_2\text{CH}=\text{CH}} = 5.5$ Hz (*trans*), $J_{\text{CH}_2\text{CH}=\text{CH}} = 2.0$ Hz (*cis*), NHCH₂), 4.62 (dd, 1H, $J_{2',1'} = 5.9$ Hz, $J_{2',3'} = 4.8$ Hz, H2'), 5.34 (dd, 1H, $J_{3',2'} = 4.8$ Hz, $J_{3',4'} = 7.1$ Hz, H3'), 5.90 (d, 1H, $J_{1',2'} = 5.9$ Hz, H1'), 6.40 (dt, 1H, $J_{\text{CH}=\text{CH}} = 16$ Hz, $J_{\text{CH}_2\text{CH}=\text{CH}} = 5.5$ Hz (*trans*), CH₂CH=), 6.55 (dt, 1H, $J_{\text{CH}=\text{CH}} = 16$ Hz (*trans*), $J_{\text{CH}_2\text{CH}=\text{CH}} = 1.5$ Hz (*cis*), =CHPh), 7.21 (tt, 1H, HpPh), 7.30 (dd, 2H, $J_{\text{Hm,Ho}} = 8.5$ Hz, $J_{\text{Hm,Hp}} = 7.4$ Hz, HmPh), 7.39 (dd, 2H, $J_{\text{Ho,Hm}} = 8.5$ Hz, $J_{\text{Ho,Hp}} = 1.2$ Hz, HoPh), 8.08 (br s, 1H, NH), 8.23 (s, 1H, H8), 8.36 (s, 1H, H2). ^{13}C NMR (100 MHz, DMSO- d_6): $\delta = 41.49$ (NHCH₂), 61.62 (C5'), 70.59 (C3'), 73.47 (C2'), 85.85 (C4'), 87.91 (C1'), 119.81 (C5), 126.06 (Ph), 127.26 (Ph), 128.53 (Ph), 129.94 (=CPh), 136.62 (NHCH₂C), 139.78 (C8), 148.38 (C4), 154.42 (C6). HRMS: m/z [M+H]⁺ calculated C₁₉H₂₂N₅O₄⁺ 384.1666, found 384.1661; m/z [M+Na]⁺ calculated C₁₉H₂₁N₅O₄Na⁺ 406.1486, found 406.1479.

4.2.6. N⁶-(3-Phenyl-2-propin-1-yl)-adenosine (7)

Following the procedure for preparation of **4**, condensation of **1** (435 mg, 1 mmol) with 3-phenyl-2-propin-1-ol (0.187 ml,

1.5 mmol) in the presence of Ph₃P (393 mg, 1.5 mmol) and DEAD (0.24 ml, 1.5 mmol) in THF (5 ml) with subsequent deblocking in 4 M PrNH₂ in MeOH solution (50 mmol) gave **7** as a white powder. Yield 209 mg (55%). R_f 0.04 (CH₂Cl₂–EtOH, 97:3), mp 128–130 °C. ^1H NMR (400 MHz, DMSO- d_6): $\delta = 3.56$ (ddd, 1H, $J_{5'b,4'} = 3.2$ Hz, $J_{5'b,5'a} = -12.1$ Hz, $J_{5'b,OH} = 6.8$ Hz, H5'b), 3.69 (ddd, 1H, $J_{5'a,4'} = 3.5$ Hz, $J_{5'a,5'b} = -12.1$ Hz, $J_{5'a,OH} = 5.3$ Hz, H5'a), 3.97 (ddd, 1H, $J_{4',3'} = 7.2$ Hz, $J_{4',5'a} = 3.5$ Hz, $J_{4',5'b} = 3.2$ Hz, H4'), 4.16 (dd, 1H, $J_{3',2'} = 4.7$ Hz, $J_{3',4'} = 7.2$ Hz, H3'), 4.56 (br s, 2H, NHCH₂), 4.61 (td, 1H, $J_{2',1'} = 6.1$ Hz, $J_{2',OH} = 6.1$ Hz, $J_{2',3'} = 4.7$ Hz, H2'), 5.29 (dd, 1H, $J_{5'a,OH} = 5.3$ Hz, $J_{5'b,OH} = 6.8$ Hz, 5'OH), 5.91 (d, 1H, $J_{1',2'} = 6.0$ Hz, H1'), 7.42–7.31 (m, 5H, Ph), 8.30 (br s, 2H, H8, NH), 8.40 (s, 1H, H2). ^{13}C NMR (100 MHz, DMSO- d_6): $\delta = 29.92$ (NHCH₂), 61.62 (C5'), 70.60 (C3'), 73.55 (C2'), 81.13 (=C–), 85.87 (C4'), 87.76 (–C≡), 87.93 (C1'), 119.97 (C5), 122.40 (Ph), 128.43 (Ph), 128.64 (Ph), 131.34 (Ph), 140.22 (C8), 148.76 (C4), 152.29 (C2), 153.94 (C6). HRMS: m/z [M+H]⁺ calculated C₁₉H₂₀N₅O₄⁺ 382.1510, found 382.1501; m/z [M+Na]⁺ calculated C₁₉H₁₉N₅O₄Na⁺ 404.1329, found 404.1321.

4.2.7. N⁶-(2-Phenoxyethyl)-adenosine (8)

Following the procedure for preparation of **4**, condensation of **1** (300 mg, 0.68 mmol) with 2-phenoxyethanol (0.129 ml, 1.02 mmol) in the presence of Ph₃P (269 mg, 1.02 mmol) and DEAD (0.16 ml, 1.02 mmol) in THF (5 ml) with subsequent deblocking in 4 M PrNH₂ in MeOH solution (25 mmol) gave **8** as a white foam. Yield 153 mg (59%). R_f 0.04 (CH₂Cl₂–EtOH, 97:3). ^1H NMR (400 MHz, DMSO- d_6): $\delta = 3.56$ (ddd, 1H, $J_{5'b,4'} = 4.0$ Hz, $J_{5'b,OH} = 4.8$ Hz, $J_{5'b,5'a} = -12.1$ Hz, H5'b), 3.67 (ddd, 1H, $J_{5'a,4'} = 3.5$ Hz, $J_{5'a,OH} = 4.8$ Hz, $J_{5'a,5'b} = -12.1$ Hz, H5'a), 3.88 (br s, 2H, NHCH₂), 3.97 (ddd, 1H, $J_{4',3'} = 3.5$ Hz, $J_{4',5'a} = 3.5$ Hz, $J_{4',5'b} = 4.0$ Hz, H4'), 4.25–4.10 (m, 3H, H3', CH₂OPh), 4.61 (ddd, 1H, $J_{2',1'} = 6.1$ Hz, $J_{2',3'} = 4.7$ Hz, $J_{2',OH} = 6.2$ Hz, H2'), 5.14 (d, 1H, $J_{3',OH} = 4.7$ Hz, 3'OH), 5.33 (t, 1H, $J_{5',OH} = 4.8$ Hz, 5'OH), 5.39 (d, 1H, $J_{2',OH} = 6.2$ Hz, 2'OH), 5.90 (d, 1H, $J_{1',2'} = 6.1$ Hz, H1'), 7.00–6.87 (m, 3H, HpPh, HmPh), 7.27 (t, 2H, $J_{\text{Ho,Hm}} = 8.0$ Hz, HoPh), 7.94 (br s, 1H, NH), 8.25 (s, 1H, H8), 8.37 (s, 1H, H2). ^{13}C NMR (100 MHz, DMSO- d_6): $\delta = 61.60$ (C5'), 65.66 (NHCH₂), 70.58 (C3'), 73.40 (CH₂OPh), 73.51 (C2'), 85.84 (C4'), 87.92 (C1'), 114.44 (Ph), 119.74 (C5), 120.56 (Ph), 129.45 (Ph), 139.67 (C8), 148.44 (C4), 152.28 (C2), 154.67 (C6), 158.39 (OPh). HRMS: m/z [M+H]⁺ calculated C₁₈H₂₂N₅O₅⁺ 388.1615, found 388.1612; m/z [M+Na]⁺ calculated C₁₈H₂₁N₅O₅Na⁺ 410.1435, found 410.1431.

4.2.8. N⁶-(4-Phenylbutane-1-yl)-adenosine (9)

Following the procedure for preparation of **4**, condensation of **1** (250 mg, 0.57 mmol) with 4-phenylbutane-1-ol (0.174 ml, 1.14 mmol) in the presence of Ph₃P (224 mg, 0.85 mmol) and DEAD (0.133 ml, 0.85 mmol) in THF (5 ml) with subsequent deblocking in 4 M PrNH₂ in MeOH solution (25 mmol) gave **9** as a white powder. Yield 242 mg (60%). R_f 0.18 (CH₂Cl₂–EtOH, 97:3), mp 133–134 °C. ^1H NMR (400 MHz, DMSO- d_6): $\delta = 1.57$ –1.65 (m, 4H, CH₂CH₂CH₂CH₂Ph), 2.60 (t, 2H, $J_{\text{CH}_2\text{CH}_2} = 6.8$ Hz, CH₂Ph), 3.45–3.60 (m, 3H, H5'b, NHCH₂), 3.68 (ddd, 1H, $J_{5'a,4'} = 3.4$ Hz, $J_{5'a,OH} = 4.2$ Hz, $J_{5'a,5'b} = -12.1$ Hz, H5'a), 3.97 (ddd, 1H, $J_{4',3'} = 3.4$ Hz, $J_{4',5'a} = 3.4$ Hz, $J_{4',5'b} = 3.4$ Hz, H4'), 4.15 (ddd, 1H, $J_{3',2'} = 5.2$ Hz, $J_{3',4'} = 3.4$ Hz, $J_{3',OH} = 4.6$ Hz, H3'), 4.61 (dd, 1H, $J_{2',1'} = 6.2$ Hz, $J_{2',3'} = 5.2$ Hz, H2'), 5.14 (d, 1H, $J_{3',OH} = 4.6$ Hz, 3'OH), 5.35–5.41 (m, 2H, 2'OH, 5'OH), 5.88 (d, 1H, $J_{1',2'} = 6.2$ Hz, H1'), 7.12–7.30 (m, 5H, Ph), 7.87 (br s, 1H, NH), 8.19 (s, 1H, H8), 8.32 (s, 1H, H2). ^{13}C NMR (100 MHz, DMSO- d_6): $\delta = 28.37$ (CH₂), 28.72 (CH₂), 34.84 (CH₂), 61.66 (C5'), 70.63 (C3'), 73.45 (C2'), 85.87 (C4'), 87.95 (C1'), 119.66 (C5), 125.55 (Ph), 128.14 (Ph), 128.24 (Ph), 139.55 (C8), 142.16 (Ph), 148.16 (C4), 152.30 (C2), 154.66 (C6).

HRMS: m/z [M+H]⁺ calculated C₂₀H₂₆N₅O₄⁺ 400.1979, found 400.1976; m/z [M+Na]⁺ calculated C₂₀H₂₅N₅O₄Na⁺ 422.1799, found 422.1795; m/z [M+K]⁺ calculated C₂₀H₂₅N₅O₄K⁺ 438.1538, found

438.1547.

4.2.9. *N*⁶-(3-Phenoxypropan-1-yl)-adenosine (**10**)

Following the procedure for preparation of **4**, condensation of **1** (350 mg, 0.79 mmol) with 3-phenoxypropan-1-ol (0.233 ml, 1.59 mmol) in the presence of Ph₃P (310 mg, 1.18 mmol) and DEAD (0.186 ml, 1.18 mmol) in THF (5 ml) with subsequent deblocking in 4 M PrNH₂ in MeOH solution (40 mmol) gave **10** as a white powder. Yield 116 mg (29%). R_f 0.18 (CH₂Cl₂–EtOH, 97:3). mp 113–116 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 2.07 (p, 2H, J_{CH₂CH₂} = 6.5 Hz, CH₂CH₂CH₂), 3.55 (ddd, 1H, J_{5'b,4'} = 3.2 Hz, J_{5'b,OH} = 6.9 Hz, J_{5'a,OH} = –12.1 Hz, H5'b), 3.68 (ddd, 1H, J_{5'a,4'} = 3.2 Hz, J_{5'a,OH} = 4.5 Hz, J_{5'a,5'b} = –12.1 Hz, H5'a), 3.62–3.73 (m, 2H, NHCH₂), 3.97 (ddd, 1H, J_{4',3'} = 3.2 Hz, J_{4',5'a} = 3.2 Hz, J_{4',5'b} = 3.2 Hz, H4'), 4.05 (t, J_{CH₂CH₂} = 6.5 Hz, CH₂OPh), 4.15 (ddd, 1H, J_{3',2'} = 4.7 Hz, J_{3',4'} = 3.2 Hz, J_{3',OH} = 4.5 Hz, H3'), 4.61 (ddd, 1H, J_{2',1'} = 5.9 Hz, J_{2',3'} = 4.7 Hz, J_{2',OH} = 6.1 Hz, H2'), 5.13 (d, 1H, J_{3',OH} = 4.5 Hz, 3'OH), 5.35 (dd, 1H, J_{5'a,OH} = 4.5 Hz, J_{5'b,OH} = 6.9 Hz, 5'OH), 5.38 (d, 1H, J_{2',OH} = 6.1 Hz, 2'OH), 5.89 (d, 1H, J_{1',2'} = 5.9 Hz, H1'), 6.88–7.31 (m, 5H, Ph), 7.92 (br s, 1H, NH), 8.20 (s, 1H, H8), 8.34 (s, 1H, H2). ¹³C NMR (100 MHz, DMSO-*d*₆): δ = 28.81 (CH₂CH₂CH₂), 37.07 (NHCH₂), 61.64 (C5'), 65.39 (CH₂O), 70.61 (C3'), 73.48 (C2'), 85.86 (C4'), 87.95 (C1'), 114.43 (Ph), 119.83 (C5), 120.39 (Ph), 129.38 (Ph), 139.67 (C8), 148.29 (C4), 152.30 (C2), 154.71 (C6), 158.60 (OPh).

HRMS: *m/z* [M+H]⁺ calculated C₁₉H₂₄N₅O₅⁺ 402.1772, found 402.1763; *m/z* [M+Na]⁺ calculated C₁₉H₂₃N₅O₅Na⁺ 424.1591, found 424.1583; *m/z* [M+K]⁺ calculated C₁₉H₂₃N₅O₅K⁺ 440.1331, found 440.1329.

4.3. 2'-Deoxyadenosine derivatives

4.3.1. *N*⁶-Benzyl-2'-deoxyadenosine (**11**)

A mixture of **2** (194 mg, 0.5 mmol), benzyl bromide (0.089 ml, 0.75 mmol) and DBU (0.149 ml, 1 mmol) in dry acetonitrile (5 ml) was kept at ambient temperature for 20 h. The reaction mixture was neutralized with acetic acid to pH –6.5 and evaporated in vacuum. The residual syrup was diluted with ethyl acetate (15 ml) and washed successively with brine (2 × 10 ml), 10% aqueous sodium bicarbonate (20 ml) and water (2 × 10 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated in vacuum. The residue was applied to column chromatography. The product was eluted with methylene chloride:ethanol – 98:2. Purified triacetyl compound was dissolved in 4 M PrNH₂ in MeOH solution (25 mmol) and was left for 24 h, after which the mixture was evaporated and the residue was applied to column chromatography. The column was washed with methylene chloride:ethanol – 95:5 and then eluted with methylene chloride:ethanol – 90:10 to give **11** as a white foam. Yield 93 mg (55%). R_f 0.08 (CH₂Cl₂–EtOH, 97:3). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 2.25 (ddd, 1H, J_{2'b,1'} = 5.8 Hz, J_{2'b,3'} = 2.3 Hz, J_{2'b,2'a} = –13.1 Hz, H2'b), 2.74 (ddd, 1H, J_{2'a,1'} = 7.8 Hz, J_{2'a,3'} = 5.7 Hz, J_{2'a,2'b} = –13.1 Hz, H2'a), 3.51 (ddd, 1H, J_{5'b,4'} = 6.2 Hz, J_{5'b,5'a} = –12.0 Hz, J_{5'b,OH} = 6.4 Hz, H5'b), 3.62 (ddd, 1H, J_{5'a,4'} = 3.9 Hz, J_{5'a,5'b} = –12.0 Hz, J_{5'a,OH} = 5.2 Hz, H5'a), 3.88 (ddd, 1H, J_{4',3'} = 2.3 Hz, J_{4',5'a} = 3.9 Hz, J_{4',5'b} = 6.2 Hz, H4'), 4.45–4.37 (m, 1H, H3'), 4.70 (br s, 2H, NHCH₂), 5.20 (dd, 1H, J_{OH,5'a} = 5.2 Hz, J_{OH,5'b} = 6.4 Hz, 5'OH), 5.3 (d, 1H, 3'OH), 6.35 (dd, 1H, J_{1',2'b} = 5.9 Hz, J_{1',2'a} = 7.8 Hz, H1'), 7.37–7.16 (m, 5H, Ph), 8.19 (s, 1H, H8), 8.35 (s, 1H, H2), 8.41 (br s, 1H, NH). ¹³C NMR (400 MHz, DMSO-*d*₆): δ = 154.90 (C6), 152.86 (C2), 148.61 (C4), 140.10 (C8), 128.82 (Ph), 127.58 (Ph), 127.32 (Ph), 119.55 (C5), 88.28 (C1'), 84.62 (C4'), 71.34 (C3'), 62.20 (C5'), 43.38 (NHCH₂), 39.49 (C2' overlapping with DMSO). HRMS: *m/z* [M+H]⁺ calculated C₁₇H₂₀N₅O₃⁺ 342.1561, found 342.1564; *m/z* [M+Na]⁺ calculated C₁₇H₁₉N₅O₃Na⁺ 364.1380, found 364.1377.

4.3.2. *N*⁶-(2-Phenylethyl)-2'-deoxyadenosine (**12**)

Following the procedure for preparation of **4**, condensation of **2** (380 mg, 1 mmol) with 2-phenylethanol (0.180 ml, 1.5 mmol) in the presence of Ph₃P (393 mg, 1.5 mmol) and DEAD (0.24 ml, 1.5 mmol) in THF (5 ml) with subsequent deblocking in 4 M PrNH₂ in MeOH solution (50 mmol) gave **12** as a white foam. Yield 206 mg (58%). R_f 0.06 (CH₂Cl₂–EtOH, 97:3). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 2.72 (ddd, 1H, J_{2'b,1'} = 6.1 Hz, J_{2'b,3'} = 2.9 Hz, J_{2'b,2'a} = –13.1 Hz, H2'b), 2.72 (ddd, 1H, J_{2'a,1'} = 7.3 Hz, J_{2'a,3'} = 5.6 Hz, J_{2'a,2'b} = –13.1 Hz, H2'a), 2.92 (dd, 2H, J_{CH₂-CH₂} = 7.8 Hz, J_{CH₂-CH₂} = 7.0 Hz, CH₂Ph), 3.52 (ddd, 1H, J_{5'b,4'} = 4.1 Hz, J_{5'b,5'a} = –12.0 Hz, J_{OH,5'b} = 6.4 Hz, H5'b), 3.62 (ddd, 1H, J_{5'a,4'} = 4.3 Hz, J_{5'a,5'b} = –12.0 Hz, J_{OH,5'a} = 5.1 Hz, H5'a), 3.82–3.60 (m, 2H, NHCH₂), 3.88 (ddd, 1H, J_{4',3'} = 6.6 Hz, J_{4',5'a} = 4.3 Hz, J_{4',5'b} = 4.1 Hz, H4'), 4.44–4.36 (m, 1H, H3'), 5.18 (dd, 1H, J_{OH,5'b} = 6.4 Hz, J_{OH,5'a} = 5.1 Hz, 5'OH), 5.27 (d, 1H, J_{OH,3'} = 3.9 Hz, 3'OH), 6.35 (dd, 1H, J_{1',2'b} = 6.2 Hz, J_{1',2'a} = 7.3 Hz, H1'), 7.35–7.15 (m, 5H, Ph), 7.83 (br s, 1H, NH), 8.22 (s, 1H, H8), 8.32 (s, 1H, H2). ¹³C NMR (100 MHz, CD₃OD): δ = 36.68 (CH₂Ph), 41.58 (C2'), 43.19 (NHCH₂), 63.67 (C5'), 73.07 (C3'), 87.15 (C4'), 89.91 (C1'), 121.30 (C5), 127.31 (Ph), 129.46 (Ph), 129.88 (Ph), 140.47 (Ph), 140.93 (C8), 148.86 (C4), 153.51 (C2), 156.21 (C6). HRMS: *m/z* [M+H]⁺ calculated C₁₈H₂₂N₅O₃⁺ 356.1717, found 356.1712; *m/z* [M+Na]⁺ calculated C₁₈H₂₁N₅O₃Na⁺ 378.1537, found 378.1528.

4.3.3. *N*⁶-(3-Phenylpropan-1-yl)-2'-deoxyadenosine (**13**)

Following the procedure for preparation of **4**, condensation of **2** (195 mg, 0.5 mmol) with 3-phenylpropan-1-ol (0.102 ml, 0.75 mmol) in the presence of Ph₃P (196 mg, 0.75 mmol) and DEAD (0.117 ml, 0.75 mmol) in THF (5 ml) with subsequent deblocking in 4 M PrNH₂ in MeOH solution (25 mmol) gave **13** as a white foam. Yield 106 mg (57%). R_f 0.11 (CH₂Cl₂–EtOH, 97:3). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 1.90 (p, 2H, J_{CH₂CH₂} = 7.6 Hz, CH₂CH₂CH₂), 2.26 (ddd, 1H, J_{2'b,1'} = 6.1 Hz, J_{2'b,3'} = 2.9 Hz, J_{2'b,2'a} = –13.0 Hz, H2'b), 2.64 (t, 2H, J_{CH₂CH₂} = 7.6 Hz, CH₂Ph), 2.72 (ddd, 1H, J_{2'a,1'} = 7.8 Hz, J_{2'a,3'} = 5.6 Hz, J_{2'a,2'b} = –13.0 Hz, H2'a), 3.58–3.45 (m, 3H, H5'b, NHCH₂), 3.63 (ddd, 1H, J_{5'a,4'} = 4.6 Hz, J_{5'a,5'b} = –12.0 Hz, J_{5'a,OH} = 5.0 Hz, H5'a), 3.88 (ddd, 1H, J_{4',3'} = 4.1 Hz, J_{4',5'a} = 4.6 Hz, J_{4',5'b} = 3.9 Hz, H4'), 4.45–4.38 (m, 1H, H3'), 5.19 (t, 1H, J_{OH,5'ab} = 7.0 Hz, 5'OH), 5.26 (d, 1H, 3'OH), 6.35 (dd, 1H, J_{1',2'b} = 6.1 Hz, J_{1',2'a} = 7.8 Hz, H1'), 7.30–7.10 (m, 5H, Ph), 7.85 (br s, 1H, NH), 8.18 (s, 1H, H8), 8.31 (s, 1H, H2). ¹³C NMR (100 MHz, DMSO-*d*₆): δ = 32.60, 30.79 (NHCH₂CH₂CH₂Ph), 39.50 (C2' overlapping with DMSO), 61.89 (C5'), 70.96 (C3'), 83.95 (C4'), 87.99 (C1'), 119.62 (C5), 125.63 (Ph), 128.22 (Ph), 139.21 (C8), 141.79 (Ph), 148.09 (C4), 152.29 (C2), 154.68 (C6). HRMS: *m/z* [M+Na]⁺ calculated C₁₉H₂₃N₅O₃Na⁺ 392.1693, found 392.1691; *m/z* [M+K]⁺ calculated C₁₉H₂₃N₅O₃K⁺ 408.1432, found 408.1427.

4.3.4. *N*⁶-(2-Phenoxyethyl)-2'-deoxyadenosine (**14**)

Following the procedure for preparation of **4**, condensation of **2** (195 mg, 0.5 mmol) with 2-phenoxyethanol (0.093 ml, 0.75 mmol) in the presence of Ph₃P (196 mg, 0.75 mmol) and DEAD (0.117 ml, 0.75 mmol) in THF (5 ml) with subsequent deblocking in 4 M PrNH₂ in MeOH solution (25 mmol) gave **14** as a white foam. Yield 135 mg (72%). R_f 0.04 (CH₂Cl₂–EtOH, 97:3). ¹H NMR (400 MHz, DMSO-*d*₆): δ = 2.28 (ddd, 1H, J_{2'b,1'} = 6.4 Hz, J_{2'b,3'} = 2.9 Hz, J_{2'b,2'a} = –13.1 Hz, H2'b), 2.72 (ddd, 1H, J_{2'a,1'} = 7.5 Hz, J_{2'a,3'} = 5.6 Hz, J_{2'a,2'b} = –13.1 Hz, H2'a), 3.52 (ddd, 1H, J_{5'b,4'} = 4.2 Hz, J_{5'b,5'a} = –12.0 Hz, J_{OH,5'b} = 6.5 Hz, H5'b), 3.62 (ddd, 1H, J_{5'a,4'} = 4.2 Hz, J_{5'a,5'b} = –12.0 Hz, J_{OH,5'a} = 5.1 Hz, H5'a), 3.95–3.80 (m, 2H, CH₂CH₂OPh), 3.88 (ddd, 1H, J_{4',3'} = 3.4 Hz, J_{4',5'a} = 4.2 Hz, J_{4',5'b} = 4.2 Hz, H4'), 4.18 (t, 2H, J_{CH₂CH₂} = 6.2 Hz, CH₂CH₂OPh), 4.41 (ddd, 1H, J_{3',2'a} = 5.6 Hz, J_{3',2'b} = 2.9 Hz, J_{3',4'} = 3.4 Hz, H3'), 5.15 (dd, 1H, J_{OH,5'b} = 6.5 Hz, J_{OH,5'a} = 5.1 Hz, 5'OH), 5.26 (d, 1H, J_{OH,3'} = 4.0 Hz, 3'OH), 6.35 (dd, 1H, J_{1',2'b} = 6.4 Hz, J_{1',2'a} = 7.6 Hz, H1'), 7.00–6.85 (m,

3H, HoPh, HpPh), 7.28 (dd, 2H, $J_{Hm, Ho} = 8.4$ Hz, $J_{Hm, Hp} = 7.2$ Hz, HmPh), 7.90 (br s, 1H, NH), 8.24 (s, 1H, H8), 8.35 (s, 1H, H2). ^{13}C NMR (100 MHz, DMSO- d_6): $\delta = 39.50$ (C', NHCH₂ overlapping with DMSO), 61.85 (C5'), 65.70 (CH₂O), 70.92 (C3'), 83.92 (C4'), 87.99 (C1'), 114.43 (Ph), 119.71 (C5), 120.56 (Ph), 129.45 (Ph), 139.52 (C8), 148.28 (C4), 152.27 (C2), 154.62 (C6), 158.39 (OPh). HRMS: m/z [M+H]⁺ calculated C₁₈H₂₂N₅O₄⁺ 372.1666, found 372.1659; m/z [M+Na]⁺ calculated C₁₈H₂₁N₅O₄Na⁺ 394.1486, found 394.1476.

4.4. Inosine derivatives

4.4.1. O⁶-(2-Phenylethyl)-inosine (21)

To the mixture of **18** [28, 30] (300 mg, 0.727 mmol) and 2-phenylethanol (7.5 ml, 61.8 mmol), *t*-BuOK (816 mg, 7.27 mmol) was gradually added. The mixture was stirred at room temperature for 24 h. After neutralization with AcOH (0.42 ml, 61.8 mmol), the solution was directly subjected to column chromatography. Elution with a gradient of EtOH in CH₂Cl₂ (1:100 → 1:8) gave **21** (252 mg, 93%) as a white powder. R_f 0.3 (CH₂Cl₂–EtOH, 9:1). mp 154–156 °C. ^1H NMR (400 MHz, DMSO- d_6 +D₂O): $\delta = 3.15$ (t, 2H, $J_{\text{CH}_2\text{-CH}_2} = 6.9$ Hz, CH₂Ph), 3.57 (ddd, 1H, $J_{5'b,5'a} = -12.0$ Hz, $J_{5'b,4'} = 3.6$ Hz, $J_{5'b,OH} = 5.3$ Hz, H5'b), 3.68 (ddd, 1H, $J_{5'a,5'b} = -12.0$ Hz, $J_{5'a,4'} = 3.6$ Hz, $J_{5'a,OH} = 5.3$ Hz, H5'a), 3.98 (ddd, 1H, $J_{4',5'b} = 3.6$ Hz, $J_{4',5'a} = 3.6$ Hz, $J_{4',3'} = 3.6$ Hz, H4'), 4.17 (dd, 1H, $J_{3',4'} = 3.6$ Hz, $J_{3',2'} = 4.4$ Hz, H3'), 4.58 (dd, 1H, $J_{2',3'} = 4.4$ Hz, $J_{2',1'} = 5.7$ Hz, H2'), 4.78 (t, 2H, $J_{\text{CH}_2\text{-CH}_2} = 6.9$ Hz, OCH₂), 5.10 (t, 1H, $J_{5',OH} = 5.3$ Hz, 5'OH), 5.18 (br s, 1H, 3'OH), 5.46 (br s, 1H, 2'OH), 5.98 (d, 1H, $J_{1',2'} = 5.7$ Hz, H1'), 7.15–7.37 (m, 5H, Ph), 8.53 (s, 1H, H2), 8.60 (s, 1H, H8). ^{13}C NMR (100 MHz, DMSO- d_6): $\delta = 34.50$ (CH₂Ph), 61.31 (C5'), 67.04 (OCH₂), 70.32 (C3'), 73.77 (C2'), 85.70 (C4'), 87.80 (C1'), 121.08 (C5), 126.35 (Ph), 128.35 (Ph), 128.88 (Ph), 137.96 (Ph), 142.36 (C8), 151.61 (C2), 151.89 (C4), 160.00 (C6). HRMS: m/z [M+H]⁺ calculated C₁₈H₂₁N₄O₅⁺ 373.1506, found 373.1505; m/z [M+Na]⁺ calculated C₁₈H₂₀N₄O₅Na⁺ 395.1326, found 395.1317.

4.4.2. O⁶-(3-Phenylpropan-1-yl)-inosine (22)

The synthesis was analogous to O⁶-(2-phenylethyl)-inosine (**21**) with 3-phenyl-1-propanol (3 ml, 29 mmol). Yield 140 mg (90%) as a foam. R_f 0.11 (CH₂Cl₂–EtOH, 95:5). ^1H NMR (400 MHz, DMSO- d_6): $\delta = 2.13$ (ddt, 2H, $J_{\text{CH}_2\text{-OCH}_2} = 6.6$ Hz, $J_{\text{CH}_2\text{-CH}_2\text{Ph}} = 6.6$ Hz, $J_{\text{CH}_2\text{-CH}_2\text{Ph}} = 7.9$ Hz, CH₂CH₂CH₂), 2.77 (dd, 2H, $J_{\text{CH}_2\text{-CH}_2} = 6.6$ Hz, $J_{\text{CH}_2\text{-CH}_2} = 7.9$ Hz, CH₂Ph), 3.58 (ddd, 1H, $J_{5'b,5'a} = -12.0$ Hz, $J_{5'b,4'} = 3.7$ Hz, $J_{5'b,OH} = 5.6$ Hz, H5'b), 3.68 (ddd, 1H, $J_{5'b,5'a} = -12.0$ Hz, $J_{5'a,4'} = 3.7$ Hz, $J_{5'a,OH} = 5.4$ Hz, H5'a), 3.98 (ddd, 1H, $J_{4',5'b} = 3.7$ Hz, $J_{4',5'a} = 3.7$ Hz, $J_{4',3'} = 3.7$ Hz, H4'), 4.17 (m, 1H, H3'), 4.55 (t, 2H, $J_{\text{OCH}_2\text{-CH}_2} = 6.6$ Hz, OCH₂), 4.55–4.63 (m, 1H, H2'), 5.12 (dd, 1H, $J_{5'b,OH} = 5.6$ Hz, $J_{5'a,OH} = 5.4$ Hz, 5'OH), 5.20 (br s, 1H, 3'OH), 5.48 (d, 1H, $J_{2',OH} = 5.0$ Hz, 2'OH), 5.99 (d, 1H, $J_{1',2'} = 5.7$ Hz, H1'), 7.16–7.32 (m, 5H, Ph), 8.52 (s, 1H, H8), 8.62 (s, 1H, H2). ^{13}C NMR (100 MHz, DMSO- d_6): $\delta = 29.91$ (CH₂), 31.34 (CH₂), 61.33 (C5'), 65.95 (OCH₂), 70.34 (C3'), 73.76 (C2'), 85.71 (C4'), 87.81 (C1'), 121.12 (C5), 125.86 (Ph), 128.27 (Ph), 128.34 (Ph), 141.40 (Ph), 142.34 (C8), 151.61 (C2), 151.85 (C4), 160.15 (C6). HRMS: m/z [M+Na]⁺ calculated C₁₉H₂₂N₄O₅Na⁺ 409.1482, found 409.1483; m/z [M+K]⁺ calculated C₁₉H₂₂N₄O₅K⁺ 425.1222, found 425.1226.

4.5. 5'-Deoxyadenosine derivatives

4.5.1. 5'-Chloro-5'-deoxyadenosine (36)

To a cold suspension (0 °C) of adenosine (3.0 g, 11.2 mmol) in acetonitrile (35 ml) and pyridine (22.4 mmol, 1.8 ml), thionyl chloride (4.1 ml, 56.1 mmol) was slowly added and the mixture was stirred 4 h under 0 °C. The resulting solution was kept at ambient temperature overnight. The precipitate was filtered and dissolved in H₂O/MeOH (5:50 ml) and 25% aqueous ammonia (4.7 ml) was

added to the mixture. The reaction mixture was kept at ambient temperature for 30 min and evaporated in vacuum. The residue was transferred to a glass filter, washed with cold water (2 × 20 ml) and dried in vacuum desiccator over P₂O₅. Yield 1.92 g (60%) as white crystals. R_f 0.15 (CH₂Cl₂–EtOH, 9:1 v/v). ^1H NMR (DMSO- d_6): $\delta = 3.84$ (dd, 1H, $J_{5'b-4'} = 6.3$ Hz, $J_{5'b-a} = -11.6$ Hz, H5'b), 3.94 (dd, 1H, $J_{5'a-4'} = 5.1$ Hz, $J_{5'a-b} = -11.6$ Hz, H5'a), 4.10 (ddd, 1H, $J_{4'-5'b} = 6.3$ Hz, $J_{4'-5'a} = 5.1$ Hz, $J_{4'-3'} = 4$ Hz, H4'), 4.23 (ddd, 1H, $J_{3'-4'} = 4$ Hz, $J_{3'-OH} = 5.1$ Hz, $J_{3'-2'} = 5$ Hz, H3'), 4.75 (dd, 1H, $J_{2'-3'} = 5$ Hz, $J_{2'-1'} = 5.6$ Hz, H2'), 5.41 (d, 1H, $J_{OH-3'} = 5.1$ Hz, 3'OH), 5.56 (d, 1H, $J_{OH-2'} = 6.03$ Hz, 2'OH), 5.93 (d, 1H, $J_{1'-2'} = 5.6$ Hz, H1'), 7.26 (s, 2H, NH₂), 8.15 (s, 1H, H8), 8.33 (s, 1H, H2).

4.5.2. 2',3'-Di-O-acetyl-5'-chloro-5'-deoxyadenosine (37)

To a cold solution (0 °C) of **36** (1.2 g, 4.1 mmol) in 10 ml of pyridine, acetic anhydride (0.85 ml, 9.0 mmol) was added under stirring and the mixture was kept at ambient temperature for 24 h. Subsequently, ethanol (10 ml) was added to the mixture. The resulting solution was kept at ambient temperature for 10 min and evaporated in vacuum. The residue was co-evaporated with toluene (2 × 40 ml) and ethanol (2 × 30 ml), transferred to a glass filter, washed with cold ethanol (15 ml) and dried in vacuum desiccator over P₂O₅ to give 1.21 g (80%) of 2',3'-di-O-acetyl-5'-chloro-5'-deoxyadenosine as white powder. R_f 0.23 (CH₂Cl₂–EtOH, 95:5). ^1H NMR (DMSO- d_6): $\delta = 2.02$ (s, 3H, Ac), 2.11 (s, 3H, Ac), 3.85 (dd, 1H, $J_{5'b-4'} = 4.21$ Hz, $J_{5'b-a} = -12.15$ Hz, H5'b), 3.89 (dd, 1H, $J_{5'a-4'} = 4.36$ Hz, $J_{5'a-b} = -12.14$ Hz, H5'a), 4.46 (ddd, 1H, $J_{4'-5'b} = 4.2$ Hz, $J_{4'-5'a} = 4.3$ Hz, $J_{4'-3'} = 3.7$ Hz, H4'), 5.61 (dd, 1H, $J_{3'-4'} = 3.7$ Hz, $J_{3'-2'} = 5.6$ Hz, H3'), 5.85 (dd, 1H, $J_{2'-3'} = 5.6$ Hz, $J_{2'-1'} = 6.07$ Hz, H2'), 6.20 (d, 1H, $J_{1'-2'} = 6.07$ Hz, H1'), 8.05 (s, 1H, H2), 8.28 (s, 1H, H8).

4.5.3. N⁶-Acetyl-2',3'-di-O-acetyl-5'-deoxyadenosine (3)

A mixture of **37** (400 mg, 1.08 mmol), α,α' -azobisisobutyronitrile (20 mg) and tributyltin hydride (1.46 ml, 5.4 mmol) in 20 ml of dry toluene was boiled during 2 h. The solution was evaporated in vacuum and the residue was chromatographed on a column, containing 60 ml of silica-gel. The column was washed with methylene chloride:ethanol – 95:5. The reaction product was eluted with methylene chloride:ethanol – 93:7. The fractions, containing the product, were collected and evaporated *in vacuo* to dryness. The residue was dissolved in pyridine (5 ml) and acetic anhydride (1 ml, 10.8 mmol) was added to the solution under stirring. The reaction mixture was kept at 60 °C for 4 h. The excess of acetic anhydride was neutralized by ethanol (20 ml). The reaction mixture was evaporated in vacuum, co-evaporated with toluene (2 × 40 ml) and ethanol (2 × 30 ml). The residue was dissolved in ethanol (5 ml) and imidazole (70 mg, 1.03 mmol) was added to the solution. The solution was kept at ambient temperature for 4 h after which it was diluted with water (20 ml) and poured into a separating funnel. The product was extracted by ethyl acetate (10 × 10 ml). The organic layers were separated, collected, dried over anhydrous sodium sulfate and evaporated *in vacuo* to dryness. Yield 310 mg (76%) as white foam. R_f 0.62 (methylene chloride:ethanol – 97:3). ^1H NMR (400 MHz, CDCl₃): $\delta = 1.52$ (d, 3H, $J_{\text{CH}_3-4'} = 6.4$ Hz, 5'-CH₃), 2.08 (s, 3H, Ac), 2.14 (s, 3H, Ac), 2.64 (s, 3H, Ac), 4.36 (qd, 1H, $J_{4'-\text{CH}_3} = 6.4$ Hz, $J_{4'-3'} = 5.2$ Hz, H4'), 5.40 (dd, 1H, $J_{3'-2'} = 5.3$ Hz, $J_{3'-4'} = 5.2$ Hz, H3'), 5.96 (dd, 1H, $J_{2'-1'} = 5.0$ Hz, $J_{2'-3'} = 5.3$ Hz, H2'), 6.14 (d, 1H, $J_{1'-2'} = 5.0$ Hz, H1'), 8.18 (s, 1H, H8), 8.71 (s, 1H, H2), 8.96 (br s, 1H, NH).

4.5.4. N⁶-Benzyl-N⁶-acetyl-2',3'-di-O-acetyl-5'-deoxyadenosine (38)

A mixture of **3** (282 mg, 0.75 mmol), benzyl bromide (0.178 ml, 1.5 mmol) and DBU (0.224 ml, 1.5 mmol) in dry acetonitrile (5 ml) was kept at ambient temperature for 3 days. The reaction mixture was neutralized with acetic acid to pH –6.5 and evaporated in

vacuum. The residual syrup was diluted with ethyl acetate (15 ml) and washed successively with brine (2 × 10 ml), 10% aqueous sodium bicarbonate (20 ml) and water (2 × 10 ml). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated in vacuum. The residue was chromatographed on a column containing 25 ml of silica-gel. The product was eluted with methylene chloride:ethanol – 98:2. Yield 282 mg (81%) as white foam. R_f 0.49 (CH₂Cl₂–EtOH, 98:2). ¹H NMR (400 MHz, CDCl₃): δ = 1.51 (d, 3H, $J_{\text{CH}_3-4'}$ = 6.6 Hz, 5'-CH₃), 2.08 (s, 3H, Ac), 2.14 (s, 3H, Ac), 2.34 (s, 3H, Ac), 4.34 (qd, 1H, $J_{4'-\text{CH}_3}$ = 6.6 Hz, $J_{4'-3'}$ = 5.6 Hz, H_{4'}), 5.41 (dd, 1H, $J_{3'-2'}$ = 5.4 Hz, $J_{3'-4'}$ = 5.6 Hz, H_{3'}), 5.54 (br s, 2H, CH₂Ph), 5.94 (dd, 1H, $J_{2'-1'}$ = 4.8 Hz, $J_{2'-3'}$ = 5.4 Hz, H_{2'}), 6.12 (d, 1H, $J_{1'-2'}$ = 4.8 Hz, H_{1'}), 7.14 (tt, 1H, $J_{\text{p-H-m-H}}$ = 8.5 Hz, $J_{\text{p-H-o-H}}$ = 1.3 Hz, Ph), 7.21 (dd, 2H, $J_{\text{o-H-m-H}}$ = 7.6 Hz, $J_{\text{o-H-p-H}}$ = 1.1 Hz, Ph), 7.28 (dd, 2H, $J_{\text{m-H-o-H}}$ = 7.6 Hz, $J_{\text{m-H-p-H}}$ = 8.5 Hz, Ph), 8.14 (s, 1H, H₈), 8.77 (s, 1H, H₂).

4.5.5. N⁶-Benzyl-5'-deoxyadenosine (**16**)

A solution of **38** (241 mg, 0.51 mmol) in 5 M propylamine in methanol (5 ml) was kept for 24 h at 20 °C and then concentrated in *vacuo* to dryness. The residue was evaporated with ethanol (5 × 10 ml). The product was precipitated from mixture methylene chloride:hexane:ethanol – 63:34:3 (3 ml). The precipitate was filtered, washed with mixture methylene chloride:hexane:ethanol – 63:34:3 (4 × 2 ml) and dried in a vacuum desiccator over P₂O₅ to give 153 mg (88%) of **16** as a white powder. R_f 0.32 (CH₂Cl₂–EtOH, 95:5). mp 198–201 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 1.30 (dd, 3H, $J_{\text{CH}_3-4'}$ = 4.77 Hz, $J_{\text{CH}_3-3'\text{OH}}$ = 1.4 Hz, CH₃), 3.93–4.10 (m, 2H, H_{3'}, H_{4'}), 4.60–4.83 (m, 3H, H_{2'}, CH₂Ph), 5.10 (d, 1H, $J_{2'-\text{OH}}$ = 4.61 Hz, 2'OH), 5.38 (d, 1H, $J_{3'-\text{OH}}$ = 5.25 Hz, 3'OH), 5.85 (dd, 1H, $J_{1'-2'}$ = 4.77 Hz, $J_{1'-2'\text{OH}}$ = 2.54 Hz, H_{1'}), 7.16–7.37 (m, 5H, Ph), 8.21 (s, 1H, H-8), 8.29–8.37 (m, 2H, H-2, NH). ¹³C NMR (100 MHz, DMSO-*d*₆): δ = 16.89 (C_{5'}), 42.89 (CH₂Ph), 73.05 (C_{3'}), 74.59 (C_{2'}), 79.71 (C_{4'}), 87.92 (C_{1'}), 119.51 (C₅), 126.54 (Ph), 127.08 (Ph), 128.14 (Ph), 139.82 (C₈), 140.04 (Ph), 148.79 (C₄), 152.54 (C₂), 154.47 (C₆). HRMS: m/z [M+Na]⁺ calculated C₁₇H₁₉N₅O₃Na⁺ 364.1380, found 364.1374.

4.5.6. N⁶-Isopentenyl-5'-deoxyadenosine (**15**)

The alkylation of **3** (200 mg) with isopentenyl bromide (0.232 ml, 1.06 mmol) was analogous to preparation of N⁶-benzyl-N⁶-acetyl-2',3'-di-O-acetyl-5'-deoxyadenosine (**38**). The deacetylation analogous to **16** yielded 80 mg (47% for two steps) of **15** as a white powder. R_f 0.31 (CH₂Cl₂–EtOH, 95:5). mp 117–120 °C. ¹H NMR (400 MHz, DMSO-*d*₆): δ = 1.30 (d, 3H, $J_{\text{CH}_3-\text{CH}}$ = 6.2 Hz, CH₃), 1.67 (d, 3H, $J_{\text{CH}_3-\text{CH}}$ = 0.8 Hz, Me-*trans*), 1.70 (d, 3H, $J_{\text{CH}_3-\text{CH}}$ = 0.5 Hz, Me-*cis*), 3.93–4.01 (m, 2H, H_{3'}, H_{4'}), 4.08 (br s, 2H, NHCH₂), 4.65 (ddd, 1H, $J_{2'-3'}$ = 4.6 Hz, $J_{2'-1'}$ = 4.8 Hz, $J_{2'-\text{OH}}$ = 5.6 Hz, H_{2'}), 5.10 (d, 1H, $J_{3'-\text{OH}}$ = 5.3 Hz, 3'OH), 5.30 (tq, 1H, $J_{\text{CH}_2-\text{CH}_2}$ = 6.6 Hz, $J_{\text{CH}_2-\text{CH}_3}$ = 1.3 Hz, CH =), 5.38 (d, 1H, $J_{2'-\text{OH}}$ = 5.6 Hz, 2'OH), 5.84 (d, 1H, $J_{1'-2'}$ = 4.8 Hz, H_{1'}), 7.77 (br s, 1H, NH), 8.21 (s, 1H, H₈), 8.29 (s, 1H, H₂). ¹³C NMR (100 MHz, DMSO-*d*₆): δ = 17.79 (Me), 18.86 (Me), 25.33 (C_{5'}), 37.67 (NHCH₂), 73.05 (C_{3'}), 74.57 (C_{2'}), 79.63 (C_{4'}), 87.84 (C_{1'}), 119.52 (C₅), 122.13 (CH =), 133.11 (CMe₂), 139.53 (C₈), 148.43 (C₄), 152.53 (C₂), 154.30 (C₆). HRMS: m/z [M+Na]⁺ calculated C₁₅H₂₁N₅O₃Na⁺ 342.1537, found 342.1534.

4.5.7. N⁶-(2-Phenylethyl)-5'-deoxyadenosine (**17**)

A mixture of **3** (322 mg, 0.85 mmol), Ph₃P (334 mg, 1.27 mmol) and 2-phenylethanol (0.153 ml, 1.27 mmol) in THF (5 ml) was stirred at ambient temperature until a homogenous solution was formed. DEAD (0.200 ml, 1.27 mmol) was added in one portion. The reaction was monitored by TLC. After 20 h, a second addition of reagents (Ph₃P, 2-phenylethanol and DEAD) in the indicated quantities was made to achieve full conversion of initial N⁶-acetyl-2',3'-di-O-acetyl-5'-deoxyadenosine. After 4–5 h, the mixture was evaporated and the residue was applied to column chromatography on silica-gel (35 ml). The product was eluted with methylene chloride:ethanol

– 97:3 (100 ml). The fractions, containing the product, were evaporated in vacuum. The residue was dissolved in 5 M propylamine in methanol (8.5 ml) and the solution was kept for 24 h at 20 °C and then concentrated in *vacuo* to dryness. The residue was evaporated with ethanol (5 × 10 ml) and applied to column chromatography on silica-gel (25 ml). The column was washed with methylene chloride:ethanol – 97:3 (50 ml), the product was eluted with methylene chloride:ethanol – 9:1. Yield 129 mg (43%) as white powder; R_f 0.30 (CH₂Cl₂–EtOH, 95:5). mp 161–163 °C. ¹H NMR (DMSO-*d*₆): δ = 1.31 (d, 3H, $J_{\text{CH}_3-4'}$ = 6.04 Hz, CH₃), 2.93 (t, 2H, $J_{\text{CH}_2-\text{CH}_2}$ = 7 Hz, CH₂Ph), 3.72 (m, 2H, NHCH₂), 3.92–4.02 (m, 2H, H_{3'}, H_{4'}), 4.66 (ddd, 1H, $J_{2'-1'}$ = 4.9 Hz, $J_{2'-3'}$ = 4.5 Hz, $J_{2'-\text{OH}}$ = 5 Hz, H_{2'}), 5.10 (d, 1H, $J_{2'-\text{OH}}$ = 5.1 Hz, 2'OH), 5.38 (d, 1H, $J_{3'-\text{OH}}$ = 5.57 Hz, 3'OH), 5.85 (d, 1H, $J_{1'-2'}$ = 4.9 Hz, H_{1'}), 7.15–7.40 (m, 5H, Ph), 7.79 (br s, 1H, NH), 8.24 (s, 1H, H₈), 8.30 (s, 1H, H₂). ¹³C NMR (DMSO-*d*₆): δ = 18.88 (C_{5'}), 35.01 (NHCH₂), 41.25 (CH₂Ph), 73.07 (C_{3'}), 74.59 (C_{2'}), 79.67 (C_{4'}), 87.85 (C_{1'}), 119.54 (C₅), 125.98 (Ph), 128.25 (Ph), 128.62 (Ph), 139.62 (C₈, Ph), 148.61 (C₄), 152.58 (C₂), 154.50 (C₆). HRMS: m/z [M+Na]⁺ calculated C₁₈H₂₁N₅O₃Na⁺ 378.1537, found 378.1526.

5. Antiviral assay

RD (rhabdomyosarcoma) cells were obtained from the European Collection of Cell Cultures and were maintained in minimal essential medium (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% sodium bicarbonate (Gibco) and 1% L-glutamine (Gibco). Cells were grown at 37 °C and 5% CO₂. EV71 strains that originated from Taiwan were a generous gift from Dr. Shih-Cheng Chang (Chang Gung University, Taoyuan, Taiwan). Strain H08300 461 #812 was acquired from National Collection of Pathogenic Viruses (NCPV). Strain 11316 was a kind gift from the National Institute for Public Health and the Environment (RIVM, The Netherlands). EV71 strain BrCr was kindly provided by F. van Kuppeveld (UMCU, The Netherlands).

The antiviral assay for EV7 was performed as described by Tijmsa et al. [28] Briefly, RD cells, grown to confluence in 96-well microtiter plates were infected with ~100 CCID₅₀ of EV71 and were treated with a dilution series of the different compounds. Cultures were incubated for three days at 37 °C (5% CO₂) after which residual cell viability was quantified using an MTS readout method according to the manufacturers' instructions (Promega).

CC₅₀ and IC₅₀ values were calculated using a custom-configured software platform from Accelrys.

Acknowledgments

This work was supported by the Russian Foundation for Basic Research (14-04-00835_a and 16-04-01594_a) and Russian Academy of Sciences (Program 'Molecular and Cell Biology'). Liang Sun is supported by a CSC: Project of Europe grant from the China Scholarship Council (CSC NO. 201403250056) and Aloys Tijmsa is supported by the European Union 7th Framework Program EUVIRNA Marie Curie Initial Training Network (264286). Kim Donckers and Caroline Collared are acknowledged for their excellent assistance in the acquisition of the antiviral data.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmech.2016.01.036>.

References

- [1] L.Y. Chang, T.Y. Lin, K.H. Hsu, Y.C. Huang, K.L. Lin, C. Hsueh, S.R. Shih, H.C. Ning, M.S. Hwang, H.S. Wang, C.Y. Lee, Clinical features and risk factors of

- pulmonary oedema after enterovirus-71-related hand, foot, and mouth disease, *Lancet* 354 (1999) 1682–1686.
- [2] K.T. Wong, B. Munisamy, K.C. Ong, H. Kojima, N. Noriyo, K.B. Chua, B.B. Ong, K. Nagashima, The distribution of inflammation and virus in human enterovirus 71 encephalomyelitis suggests possible viral spread by neural pathways, *J. Neuropathol. Exp. Neurol.* 67 (2008) 162–169.
- [3] W. Xing, Q. Liao, C. Viboud, J. Zhang, J. Sun, J.T. Wu, Z. Chang, F. Liu, V.J. Fang, Y. Zheng, B.J. Cowling, J.K. Varma, J.J. Farrar, G.M. Leung, H. Yu, Hand, foot, and mouth disease in China, 2008–12: an epidemiological study, *Lancet Infect. Dis.* 14 (2014) 308–318.
- [4] P.N. Huang, S.R. Shih, Update on enterovirus 71 infection, *Curr. Opin. Virology* 5 (2014) 98–104.
- [5] P. Chong, S.Y. Hsieh, C.C. Liu, A.H. Chou, J.Y. Chang, S.C. Wu, S.J. Liu, Y.H. Chow, I.J. Su, M. Klein, Production of EV71 vaccine candidates, *Hum. Vaccines Immunother.* 8 (2012) 1775–1783.
- [6] L. Shang, M. Xu, Z. Yin, Antiviral drug discovery for the treatment of enterovirus 71 infections, *Antivir. Res.* 97 (2013) 183–194.
- [7] H.R. Pourianfar, L. Grollo, Development of antiviral agents toward enterovirus 71 infection, *Journal of Microbiology, Immunol. Infect.* 48 (2015) 1–8.
- [8] C.W. Tan, J.K.F. Lai, I.C. Sam, Y.F. Chan, Recent developments in antiviral agents against enterovirus 71 infection, *J. Biomed. Sci.* 21 (2014) 1–11.
- [9] L.Q. Shang, M.Y. Xu, Z. Yin, Antiviral drug discovery for the treatment of enterovirus 71 infections, *Antivir. Res.* 97 (2013) 183–194.
- [10] R.L. Kuo, S.R. Shih, Strategies to develop antivirals against enterovirus 71, *Virology J.* 10 (2013) 28.
- [11] H.J. Thibaut, A.M. De Palma, J. Neyts, Combating enterovirus replication: State-of-the-art on antiviral research, *Biochem. Pharmacol.* 83 (2012) 185–192.
- [12] M. Arita, T. Wakita, H. Shimizu, Characterization of pharmacologically active compounds that inhibit poliovirus and enterovirus 71 infectivity, *J. Gen. Virol.* 89 (2008) 2518–2530.
- [13] L. Shang, Y. Wang, J. Qing, B. Shu, L. Cao, Z. Lou, P. Gong, Y. Sun, Z. Yin, An adenosine nucleoside analogue NITD008 inhibits EV71 proliferation, *Antivir. Res.* 112 (2014) 47–58.
- [14] V.I. Tararov, A. Tijmsa, S.V. Kolyachkina, V.E. Oslovsky, J. Neyts, M.S. Drenichev, P. Leyssen, S.N. Mikhailov, Chemical modification of the plant isoprenoid cytokinin N6-isopentenyladenosine yields a selective inhibitor of human enterovirus 71 replication, *Eur. J. Med. Chem.* 90 (2015) 406–413.
- [15] B.B. Mishra, V.K. Tiwari, Natural products: An evolving role in future drug discovery, *Eur. J. Med. Chem.* 46 (2011) 4769–4807.
- [16] G.M. Cragg, D.J. Newman, Natural products: A continuing source of novel drug leads, *Biochim. Biophys. Acta* 1830 (2013) 3670–3695.
- [17] R. Mah, J.R. Thomas, C.M. Shafer, Drug discovery considerations in the development of covalent inhibitors, *Bioorg. Med. Chem. Lett.* 24 (2014) 33–39.
- [18] J. Zeidler, D. Baraniak, T. Ostrowski, Bioactive nucleoside analogues possessing selected five-membered azaheterocyclic bases, *Eur. J. Med. Chem.* 97 (2015) 409–417.
- [19] Z. Jahnz-Wechmann, G. Framski, P. Januszczuk, J. Boryski, Bioactive fused heterocycles: Nucleoside analogs with an additional ring, *Eur. J. Med. Chem.* 97 (2015) 388–396.
- [20] V.I. Tararov, S.V. Kolyachkina, C.S. Alexeev, S.N. Mikhailov, N6-Acetyl-2',3',5'-tri-O-acetyladenosine – a convenient, missed out substrate for regioselective N6-alkylations, *Synthesis* (2011) 2483–2489.
- [21] M.H. Fleysher, A. Bloch, M.T. Hakala, C.A. Nichol, Synthesis and biological activity of some new N⁶-substituted purine nucleosides, *J. Med. Chem.* 12 (1969) 1056–1061.
- [22] K. Doležal, I. Popa, E. Hauserová, L. Spíchal, K. Chakrabarty, O. Novák, V. Krystof, J. Voller, J. Holub, M. Strnad, Preparation, biological activity and endogenous occurrence of N⁶-benzyladenosines, *Bioorg. Med. Chem.* 15 (2007) 3737–3747.
- [23] S.N. Mikhailov, N.B. Grishko, Acyclic analogs of nucleosides. Synthesis of chiral 1,5-dihydroxy-4-methyl-3-oxapent-2-yl derivatives of uracyl, *Khimiya Geterotsiklicheskikh Soedinenii* (1988) 91–94.
- [24] M. Ikehara, S. Uesugi, M. Kaneko, part 2, in: L.B. Townsend, R.S. Tipson (Eds.), *Nucleic Acid Chemistry*, Wiley, New York, 1970, p. 610.
- [25] S. Casati, A. Manzocchi, R. Ottria, P. Ciuffreda, ¹H, ¹³C and ¹⁵N NMR assignments for N⁶-isopentenyladenosine/inosine analogues, *Magn. Res. Chem.* 48 (2010) 745–748.
- [26] J. Vicha, M. Maloň, P. Veselá, O. Humpa, M. Strnad, R. Marek, ¹H-, ¹³C-, and ¹⁵N-NMR chemical shifts for selected glucosides and ribosides of aromatic cytokinins, *Magn. Res. Chem.* 48 (2010) 318–322.
- [27] S.V. Kolyachkina, V.I. Tararov, C.S. Alexeev, D.M. Krivosheev, G.A. Romanov, E.V. Stepanova, E.S. Solomko, A.N. Inshakov, S.N. Mikhailov, N6-Substituted adenosines. Cytokinin and antitumor activities, *Collect. Czech. Chem. Commun.* 76 (2011) 1361–1378.
- [28] P. Franchetti, L. Cappellacci, P. Vita, R. Petrelli, A. Lavecchia, S. Kachler, K.N. Klotz, I. Marabese, L. Luongo, S. Maione, M. Grifantini, N6-Cycloalkyl- and N6-bicycloalkyl-C5'(C2')-modified adenosine derivatives as high-affinity and selective agonists at the human A1 adenosine receptor with antinociceptive effects in mice, *J. Med. Chem.* 52 (2009) 2393–2406.
- [29] J. Hsiao, M.-Y. Shen, K.-H. Lin, C.-Y. Chou, N.-H. Tzu, C.-H. Lin, D.-S. Chou, T.-F. Chen, J.-R. Sheu, Inhibitory activity of kinetin on free radical formation of activated platelets in vitro and on thrombus formation in vivo, *Eur. J. Pharmacol.* 465 (2003) 281–287.
- [30] A. Dassano, M. Mancuso, P. Giardullo, L. De Cecco, P. Ciuffreda, E. Santaniello, A. Saran, T.A. Dragani, F. Colombo, N6-Isopentenyladenosine and analogs activate the NRF2-mediated antioxidant response, *Redox Biol.* 2 (2014) 580–589.
- [31] L. Sáenz, L.H. Jones, C. Oropeza1, D. Vlácil, M. Strnad, Endogenous isoprenoid and aromatic cytokinins in different plant parts of *Cocos nucifera* (L.), *Plant Growth Regul.* 39 (2003) 205–215.
- [32] P. Mlejnek, P. Dolezel, S. Prochazka, Intracellular conversion of cytokinin bases into corresponding mononucleotides is related to cell death induction in tobacco BY-2 cells, *Plant Sci.* 168 (2005) 389–395.
- [33] P. Nauš, R. Pohl, I. Vortuba, P. Džubák, M. Hujdúch, R. Ameral, G. Birkuš, T. Wang, A.S. Ray, R. Mackman, T. Cihlar, M. Hocek, 6-(Het)aryl-7-deazapurine ribonucleosides as novel potent cytostatic agents, *J. Med. Chem.* 53 (2010) 460–470.
- [34] H.E. Gottlieb, V. Kotlyar, A. Nudelman, NMR chemical shifts of common laboratory solvents as trace impurities, *J. Org. Chem.* 62 (1977), 7512–7215.
- [35] P.A. Belyakov, V.I. Kadentsev, A.O. Chizhov, N.G. Kolotyrykina, A.S. Shashkov, V.P. Ananikov, Mechanistic insight into organic and catalytic reactions by joint studies using mass spectrometry and NMR spectroscopy, *Mendeleev Commun.* 20 (2010) 125–131.
- [36] L.W. McLaughlin, N. Piel, T. Hellman, Preparation of protected ribonucleosides suitable for chemical oligoribonucleotide synthesis, *Synthesis* (1985) 322–323.
- [37] S.N. Mikhailov, V.L. Florentiev, W. Pfeleiderer, Convenient synthesis of partially blocked oxidized-reduced nucleosides, *Synthesis* 4 (1985) 399–400.
- [38] A. Mittelman, J. Evans, G. Chheda, Cytokinins as chemotherapeutic agents, *Ann. N.Y. Acad. Sci.* 255 (1976) 225–234.