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Expeditious synthesis and biological evaluation of new C-6 1,2,3-triazole adenosine derivatives A1 receptor antagonists or agonists†

Smitha C. Mathew,[a] Youlet By,[b] Aurélie Berthault,[a] Marie-Alice Virolleaud,[a] Louis Carrega,[b] Gaëlle Chouraqui,[a] Laurent Commeiras,[a] Jocelyne Condo,[b] Mireille Attolini,[a] Anouk Gaudel-Siri,[a] Jean Ruf,[b] Jean Rodriguez,*[a] Jean-Luc Parrain*[a] and Régis Guieu*[b,c]

The synthesis of new C-6 1,2,3-triazole adenosine derivatives *via* microwave assisted 1,3-dipolar cycloaddition as key step is described. The binding on membranes of cells that over express A_1 adenosine receptors (A_1AR) was also evaluated. Among them, four compounds increased cAMP production, in a dose-dependent manner acting as antagonists of the A_1AR, while two compounds act as agonists.

Introduction

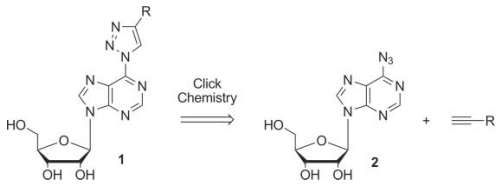
Adenosine, an ubiquitous nucleoside that comes from the dephosphorylation of ATP, acts on G protein-coupled adenosine receptors (AR), named A_1, A_{2A}, A_{2B} and A_3, depending on the pharmacological properties of the receptor subtypes.[1] Each AR has a specific tissue distribution, ligand affinity, and signal transduction mechanism. Because of their presence on nearly every cell type, adenosine receptors have been obvious targets for the development of new drugs for more than three decades.[2] The A_1 adenosine receptors (A_1AR), highly expressed in heart, brain, dorsal spinal cord, adipose tissue and kidney,[3] particularly raised our attention. According to the recent literature the best approach for the discovery of new AR agonists consists of N^6 modifications or introduction of selective functionalization at 2-position of the adenine moiety and at the 3′-, 4′- or 5′-position of the ribose part. Among them N^6-chlorocyclopentyladenosine (CCPA) and N^6-cyclopentyladenosine (CPA) have been reported as being highly selective A_1AR agonists.[4] On the other hand, reported AR antagonists are usually derivatives of xanthines such as caffeine or theophylline.[5] Thus, having drugs that modulate A_1AR at one's disposal could be interesting in different therapeutic areas, including heart failure, diuresis,[6] heart arrhythmia,[7] glucose tolerance,[8] cognitive diseases[9] and contrast media-induced acute renal failure.[10]

Nevertheless, the modified adenosine approach is hampered by the presence of adenosine deaminase (ADA) involved in purines metabolism that irreversibly converts adenosine to the less active inosine nucleoside by selective C-6 deamination. To circumvent this drawback, the design of new analogues resistant to ADA bearing a non metabolisable C-6 amino group is needed. In this context, we propose an expeditious approach based on click-chemistry of specifically functionalized nucleoside analogues starting from commercially available inosine.

New methods for the synthesis of C6 purine derivatives have been widely developed for two decades. Advances in this field employ nucleophilic displacement (S_NAr),[11] and the area of organometallic cross-coupling[12] such as Suzuki–Miyaura,[13] Sonogashira,[14] Stille,[15] and Buchwald–Hartwig[16] coupling reactions. Alternatively, the discovery[17] of the dramatic acceleration of the copper(I) mediated Huisgen 1,3-dipolar cycloaddition of alkyne and azides to yield 1,2,3-triazoles has generated the synthesis of a plethora of new compounds and has accelerated advances in areas ranging from drug discovery to material sciences.[18] Although a number of 1,2,3-triazole nucleoside derivatives has been described,[19] most involve introduction of 1,2,3-triazole at C2,[20] and C8[21] or at the sugar moiety.[22] To the best of our knowledge, when we started the project, this "click reaction" was unexploited for the synthesis of N^6-aminopurine derivatives.[23] As part of our ongoing research towards the synthesis of biologically active adenosine derivatives,[24] we have developed this approach for a rapid access to new N^6 nucleoside derivatives. We describe herein the synthesis, the A_1 binding affinities and the biological evaluation of a novel series of new C-6 1,2,3-triazole substituted purine derivatives **1** *via* microwave assisted 1,3-dipolar cycloaddition.

Results and discussion

As outlined retrosynthetically in Scheme 1, C-6 triazole substituted adenosines **1** could be readily obtained by "click chemistry" between C-6 azido adenosine **2** and different substituted alkynes. Our investigation started with the synthesis of 1,3-dipolar partner **2** (Scheme 2). As described in the literature,[25,26] C-6 azido



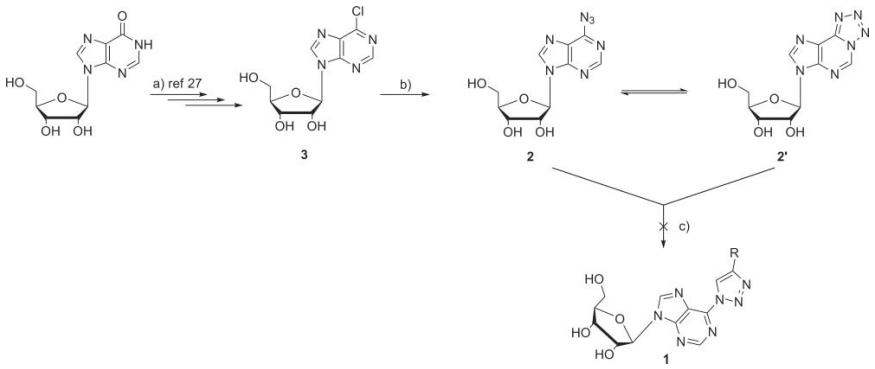
Scheme 1 Retrosynthetic strategy for the synthesis of **1**.

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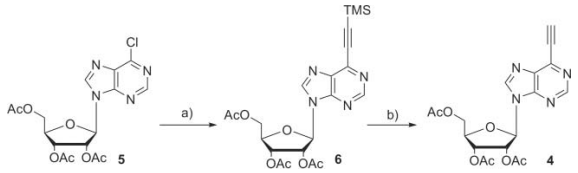


Scheme 2 Preparation of C-6 azido adenosine **2**. *Reagents and conditions*: (a) three steps, 90% overall yield; (b) LiN_3, DMF, rt, 2 days (30%); (c) phenylacetylene, conditions A: $CuSO_4{\cdot}5H_2O$, sodium ascorbate, DMF, 80 °C, 12 h (45% of adenosine), conditions B: $CuSO_4{\cdot}5H_2O$, sodium ascorbate, DMF, MW, 100 °C, 30 min (80% of adenosine), conditions C: $CuSO_4{\cdot}5H_2O$, sodium ascorbate, tBuOH : H_2O, rt, 2 h (75% of adenosine) or conditions D: Cu wire, CH_3CN–H_2O, 35 °C, 12 h (65% of adenosine).

adenosine **2** can be synthesized *via* nucleophilic displacement reaction of the electrophilic nucleoside **3** by sodium azide. 6-Chloropurine riboside (**3**) was prepared in three steps (90% overall yield) according to a previously reported procedure from the commercially available inosine.[27]

However, the S_NAr reaction of **3** using the reported conditions did not furnish the corresponding azide **2**. Despite numerous attempts to modify the reaction conditions (lower temperature, microwave irradiation or protection of the alcohol function on the sugar part as an acetate or acetal), **2** was always detected only in trace amount. Finally replacement of sodium azide by lithium azide (DMF, rt, 2 days)[28] led, in 30% yield, to the formation of **2** in equilibrium with its tetrazolo tautomeric form **2′**.[25,28,29]

Even if **2** was obtained in low yield, the copper(I)-mediated Huisgen 1,3-dipolar cycloaddition was then investigated (Scheme 2). Phenylacetylene was chosen as a benchmark substrate and the reaction was initially performed with sodium ascorbate, $CuSO_4{\cdot}5H_2O$ in DMF for 12 h at 80 °C (conditions A). Unfortunately, the desired 1,4-disubstituted 1,2,3-triazole **1** (R=Ph) was never observed. Adenosine, resulting from the reduction of the azido function of **2** into the corresponding amine, was isolated in 45% yield as the main reaction product. This kind of reduction was already described by Frieden *et al.* and can be explain by a thermal decomposition of the azido function to the corresponding nitrene, which evolved into the amine formation.[25] Whatever the conditions used to perform the 1,3-dipolar cycloaddition (Scheme 2, conditions B: $CuSO_4{\cdot}5H_2O$, sodium ascorbate, DMF, MW, 100 °C, 30 min, conditions C: $CuSO_4{\cdot}5H_2O$, sodium ascorbate, tBuOH : H_2O, rt, 2 h or conditions D: Cu wire, CH_3CN–H_2O, 35 °C, 12 h), adenosine was always the sole product obtained (65–80%). The presence of the C-6 tretrazolyl tautomer could explain the lack of reactivity in the [3+2]-cycloaddition process. This observation drove us to envision a new strategy. Instead of installing the azide function on the C-6 position of the adenosine moiety, it was decided to prepare the corresponding C-6 acetylene **4**. The readily available protected chloroinosine **5** (Scheme 3) underwent a smooth Sonogashira coupling with TMS-acetylene. The installation of the triple bond at the C-6 position of the adenosine derivative was accomplished in 69% yield.[30] Deprotection of the TMS group was performed



Scheme 3 Synthesis of **4**. *Reagents and conditions*: (a) $PdCl_2(PPh_3)_2$, CuI, NEt_3, DMF, 60 °C, 3 h (69%); (b) TBAF, THF, 0 °C, 20 min (43%).

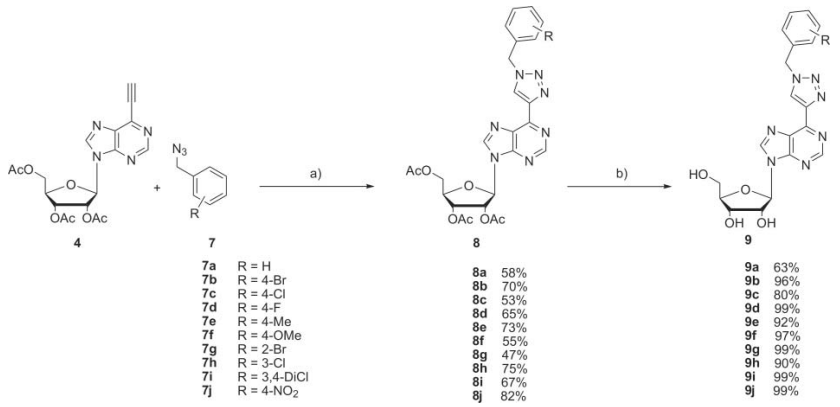
with tetrabutylammonium fluoride[31] to yield the terminal alkyne **4** in 43% yield.

The Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction was next studied. In an initial experiment (Scheme 4), the alkynyl derivative **4** was treated with benzyl azide **7a** in dry DMF at 90 °C in the presence of copper(II) and sodium ascorbate under microwave conditions for 30 min. We were pleased to find that these conditions afforded the triazole derivative **8a** in 58% yield thus validating this new approach (Scheme 4). In order to examine the scope of this approach, the [3+2]-cycloaddition was then performed with a range of different azides **7b–j** (Scheme 4). The latter were prepared according to a safe literature procedure developed by Varma *et al.*[32] based on a nucleophilic substitution reaction of alkyl halides by sodium azide in water using microwave-assisted heating. The 1,3-dipolar cycloaddition has then allowed the formation of several new C-6 1,2,3-triazole adenosine derivatives **8b–j** in good yields (up to 82%) and with a total regioselectivity.

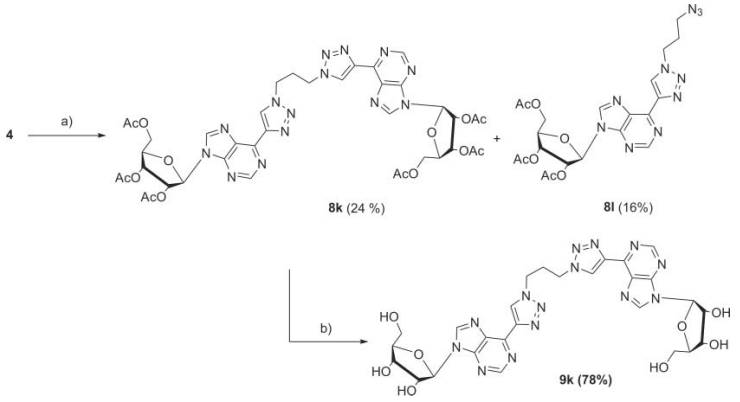
Interestingly, when the reaction was performed with 1,3-diazidopropane **7k**, a separable mixture of bis-C6-1,2,3-triazole bis-adenosine **8k** and mono cycloadduct derivative **8l** were obtained in moderate yield (respectively 24 and 16%, Scheme 5). At last, deprotection of the acetate functions of the sugar part under basic condition (NH_3, MeOH) led to the formation of the corresponding compounds **9a–k** in excellent yields (up to 99%, Scheme 4 and 5).

Biological evaluation

These new C-6 1,2,3-triazole adenosine derivatives **9a–k** were then submitted to biological evaluation. The ability of these



Scheme 4 Preparation of C-6 1,2,3-triazole substituted adenosines **9**. *Reagents and conditions*: (a) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF, μW, 90 °C, 30 min; (b) NH_3, MeOH, rt, 12 h.



Scheme 5 Reactivity of **4** with 1,3-diazidopropane. (a) $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF, μW, 90 °C, 30 min, 1,3-diazodopropane **7k**; (b) NH_3, MeOH, rt, 12 h.

unprecedented molecules to modulate cAMP production in mammalian Chem-3 cells that over expressed A_1ARs was first tested. Indeed, agonists of A_1AR are believed to decrease the cAMP production whereas antagonists display the opposite effect.

As a matter of fact **9e**, **9f**, **9h** and **9i** increased cAMP production in cell culture, in a dose-dependent manner acting as antagonists of the A_1AR (Table 1), while **9j** and **9k** decreased cAMP production acting as agonists (Table 1). The IC_{50} of compounds was also evaluated, using binding assay on cell membranes. IC_{50} of antagonist compounds range between 0.29 μM and 1.5 μM and between 11 μM and 42 μM for agonist compounds (Table 1). Even if these molecules have weaker affinity when compared to the well known A_1AR antagonist DPCPX, their biological effects occur at reasonable concentrations.

Compounds **9a**, **9b**, **9c**, **9d** and **9g** had an IC_{50} for A1AR binding >100 μM and all compounds tested had an IC_{50} for A_{2A} adenosine receptor binding >50 μM (data not shown). While nitroaromatic compounds have been reported to have A_1AR antagonist properties,[33] interestingly, **9j** exhibits agonist properties. It is also interesting to note that nitroaromatic compounds were known to act *via* the inhibition of NO synthase and cGMP modulation.[34]

Conclusions

In summary we have reported the synthesis of new C-6 1,2,3-triazole adenosine derivatives *via* microwave assisted 1,3-dipolar cycloaddition as key step. Among them, four compounds expressed antagonist properties, while two expressed agonist properties, basing on cAMP production on A_1AR. Their biological effects were obtained at reasonable concentrations.

Experimental

All reactions sensitive to oxygen and moisture were carried out in oven-dried glassware under a slight positive pressure of argon unless otherwise noted. Reagents and solvents were commercial grades and were used as supplied. *N*,*N*-dimethylformamide was distilled from calcium hydride and stored over calcium hydride. THF was obtained from a Solvent Purification System. Triethylamine was distilled from KOH prior to use. Analytical thin layer chromatography (TLC) was performed on Merck precoated analytical plates, 0.25 mm thick, silica gel 60 F254. Flash column chromatography was performed on Merck Kieselgel 40–63 mm. ^{1}H NMR and ^{13}C NMR spectra were recorded on an AC300 using the deuterated solvent as internal deuterium lock. Chemical

Table 1 Effects of compounds on cAMP production and on [^{3}H] DPCPX displacement or [^{3}H] CPA displacement on Chem 3 cells that overexpress the A1 AR. IC_{50} for cAMP inhibition was defined as the concentration of compound that induces half of the maximal cAMP inhibition. EC_{50} for cAMP production was defined as the concentration of compound that induces half of the maximal cAMP production. IC_{50} for DPCPX or CPA displacement was defined as the concentration of competing ligand which displaces 50% of [^{3}H] DPCPX or [^{3}H] CPA used as specific radioligand

	cAMP production		[^{3}H]DPCPX displacement	[^{3}H]CPA displacement	
Compound	EC_{50}/M	IC_{50}/M	IC_{50}/M	IC_{50}/M	Properties
9a			$>10^{-5}$		—
9b			$>10^{-5}$		—
9c			$>10^{-5}$		—
9d			$>10^{-5}$		—
9e	$5.5 \pm 0.9\ 10^{-7}$		$2.9 \pm 0.8\ 10^{-7}$		Antagonist
9f	$7 \pm 2\ 10^{-7}$		$15 \pm 4\ 10^{-7}$		Antagonist
9g			$>10^{-5}$		—
9h	$6 \pm 2\ 10^{-6}$		$6 \pm 1.8\ 10^{-7}$		Antagonist
9i	$7 \pm 2\ 10^{-7}$		$4 \pm 2.9\ 10^{-7}$		Antagonist
9j		$1 \pm 0.6\ 10^{-6}$		$42 \pm 11\ 10^{-6}$	Agonist
9k		$0.5 \pm 0.3\ 10^{-6}$		$11 \pm 4\ 10^{-6}$	Agonist
DPCPX	$3.8 \pm 0.5\ 10^{-9}$		$4 \pm 0.8\ 10^{-9}$		Antagonist

shift data are given in units δ relative to residual protic solvent where δ(chloroform) = 7.26 ppm, δ(DMSO) = 2.50 ppm and δ(methanol) = 3.31 ppm The multiplicity of a signal is indicated as: br – broad, s – singlet, d – doublet, t – triplet, q – quartet, m – multiplet, dd – doublet of doublets, *etc.* Coupling constants (J) are quoted in Hz and recorded to the nearest 0.1 Hz. ^{13}C NMR spectra were recorded on an AC300 spectrometer using the deuterated solvent as internal deuterium lock. Chemical shift data are given in units δ relative to residual protic solvent where δ(chloroform) = 77.16 ppm, δ(DMSO) = 39.52 ppm and δ(methanol) = 49.00 ppm. High resolution mass spectra (HRMS) have been performed using a mass spectrometer equipped with a pneumatically assisted atmospheric pressure ionization. The sample was ionized in positive mode electrospray.

(2*R*,3*R*,4*S*,5*R*)-2-(6-azido-9*H*-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol 2

To a stirred solution of chloroinosine **3** (400 mg, 1.38 mmol) in dry DMF was added lithium azide (750 mg, 15.2 mmol). The reaction was stirred at room temperature for 60 h then the solvent was removed under vacuum. The crude product was recrystallized from methanol to give **2** (120 mg, 30%). δ_H(300 MHz, DMSO) 10.14 (s, 1H), 8.93 (s, 1H), 6.15 (d, J = 5.1 Hz, 1H), 5.66 (d, J = 5.9 Hz, 1H), 5.33 (d, J = 5.3 Hz, 1H), 5.14 (appt, J = 5.3 and 5.5 Hz, 1H), 4.57 (q, J = 5.3 Hz, 1H), 4.21 (q, J = 4.7 Hz, 1H), 4.02 (q, J = 4.0 Hz, 1H), 3.76–3.69 (m, 1H), 3.65–3.57 (m, 1H); δ_C(75 MHz, DMSO) 145.4 (C), 142.6 (CH), 142.0 (C), 136.1 (CH), 120.3 (C), 88.4 (CH), 85.8 (CH), 74.6 (CH), 70.1 (CH), 61 (CH_2).

(2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(6-((trimethylsilyl)ethynyl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate 6

To a purged argon solution of chloroinosine 3 (1.1 g, 2.6 mmol), trimethylsilyl acetylene (0.6 mL, 3.1 mmol), $PdCl_2(PPh_3)_2$ (54 mg, 0.078 mmol) and copper iodide (30 mg, 0.156 mmol) in DMF was added triethylamine (1 mL, 8.4 mmol). The mixture was then heated at 60 °C for 3 h and the solvents were evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (1/1 pet. ether–ethyl acetate) to afford **6** (800 mg, 69%). δ_H(300 MHz, $CDCl_3$) 8.85 (s, 1H), 8.24 (s, 1H), 6.17 (d, J = 5.3 Hz, 1H), 5.88 (appt, J = 5.5 and 5.3 Hz, 1H), 5.58 (br appt, J = 4.9 and 5.1 Hz, 1H), 4.41–4.26 (m, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H), 0.25 (s, 9H); δ_C(75 MHz, $CDCl_3$) 170.2 (C), 169.5 (C), 169.3 (C), 153.7 (CH), 151.3 (C), 143.9 (CH), 141.7 (C), 134.9 (C), 106.0 (C), 98.2 (C), 86.5 (CH), 80.4 (CH), 73.0 (CH), 70.6 (CH), 62.9 (CH_2), 20.7 (CH_3), 20.5 (CH_3), 20.3 (CH_3), –0.5 (3 × CH_3); HRMS (ESI) calcd for $C_{21}H_{27}N_4O_7Si$ $[M+H]^+$: 475.1644 found 475.1642

(2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(6-ethynyl-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate 4

To a stirred solution of 6 (1.5 g, 3.1 mmol) in THF (20 mL) at 0 °C was added dropwise TBAF (3.4 mL, 3.4 mmol, 1M in THF) and stirred for 20 min. The reaction mixture was quenched with saturated aqueous ammonium chloride solution and the solvent was removed under vacuum. The residue was extracted with dichloromethane, dried over sodium sulfate then concentrated. The resulting mixture was purified by column chromatography on silica gel (94/6 dichloromethane–methanol) to give **4** (550 mg, 43%). δ_H(300 MHz, $CDCl_3$) 8.78 (s, 1H), 8.25 (s, 1H), 6.12 (d, J = 5.1 Hz, 1H), 5.82 (br appt, J = 5.5 and 5.3 Hz, 1H), 5.51 (br appt, J = 5.1 and 5.3 Hz, 1H), 4.33–4.17 (m, 3H), 3.73 (s, 1H), 1.97 (s, 3H), 1.92 (s, 3H), 1.90 (s, 3H); δ_C(75 MHz, $CDCl_3$) 170.0 (C), 169.3 (C), 169.1 (C), 152.4 (CH), 151.0 (C), 144.2 (CH), 140.7 (C), 135.2 (C), 86.6 (C), 86.3 (CH), 80.1 (CH), 77.6 (CH), 72.8 (CH), 70.2 (CH), 62.7 (CH_2), 20.4 (CH_3), 20.2 (CH_3), 20.1 (CH_3); HRMS (ESI) calcd for $C_{18}H_{19}N_4O_7$ $[M+H]^+$: 403.1248 found 403.1241

General procedure for the synthesis of compounds 8a–l.

A mixture of acetylene **4** (70 mg, 0.17 mmol), benzyl azide (0.24 mmol), sodium ascorbate (10 mg, 0.051 mmol) and copper sulfate pentahydrate (2 mg) in dry DMF (2 mL) was subjected to microwave irradiation for 30 min at a temperature of 90 °C. The reaction mixture was then concentrated, extracted with dichloromethane and the residue was purified by column chromatography using dichloromethane–methanol as eluent to afford the triazole derivatives **8a–l**.

(2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(6-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate 8a. δ_H(300 MHz, $CDCl_3$) 9.00 (s, 1H), 8.64 (s, 1H), 8.24 (s, 1H), 7.40–7.31 (m, 5H), 6.25 (d, $J = 5.3$ Hz, 1H), 5.95 (t, $J = 5.5$ Hz, 1H), 5.68–5.65 (m, 3H), 4.47–4.32 (m, 3H), 2.13 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H); δ_C(75 MHz, $CDCl_3$) 170.4 (C), 169.7 (C), 169.4 (C), 153.2 (CH), 151.8 (C), 148.0 (C), 143.9 (C), 143.2 (CH), 134.4 (C), 130.3 (C), 129.3 (2 × CH), 129.0 (CH), 128.2 (2 × CH), 126.9 (CH), 86.5 (CH), 80.5 (CH), 73.2 (CH), 70.7 (CH), 63.1 (CH_2), 54.5 (CH_2), 20.8 (CH_3), 20.6 (CH_3), 20.4 (CH_3); HRMS (ESI) calcd for $C_{25}H_{26}N_7O_7$ $[M+H]^+$: 536.1888 found 536.1878.

(2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(6-(1-(4-bromobenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate 8b. δ_H(300 MHz, $CDCl_3$) 8.99 (s, 1H), 8.65 (s, 1H), 8.24 (s, 1H), 7.48 (d, $J = 8.5$ Hz, 2H), 7.18 (d, $J = 8.5$ Hz, 1H), 6.24 (d, $J = 5.1$ Hz, 1H), 5.95 (appt, $J = 5.5$ and 5.3 Hz, 1H), 5.66 (br dd, $J = 5.3$ and 4.5 Hz, 1H), 5.60 (s, 2H), 4.47–4.28 (m, 3H), 2.12 (s, 3H), 2.08 (s, 3 H), 2.04 (s, 3 H); δ_C(75 MHz, $CDCl_3$) 170.3 (C), 169.6 (C), 169.4 (C), 153.2 (CH), 151.8 (C), 147.8 (C), 144.0 (C), 143.3 (CH), 133.4 (C), 132.4 (2 × CH), 130.3 (C), 129.8 (2 × CH), 126.9 (CH), 123.1 (C), 86.5 (CH), 80.5 (CH), 73.2 (CH), 70.7 (CH), 63.1 (CH_2), 53.7 (CH_2), 20.8 (CH_3), 20.6 (CH_3), 20.4 (CH_3); HRMS (ESI) calcd for $C_{25}H_{25}BrN_7O_7$ $[M+H]^+$: 614.0993 found 614.0994.

(2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(6-(1-(4-chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate 8c. δ_H(300 MHz, $CDCl_3$) 9.02 (s, 1H), 8.65 (s, 1H), 8.25 (s, 1H), 7.35 (d, $J = 8.7$ Hz, 2H), 7.26 (d, $J = 8.7$ Hz, 1H), 6.26 (d, $J = 5.3$ Hz, 2H), 5.96 (t, $J = 5.5$ Hz, 1H), 5.68 (br dd, $J = 5.5$ and 4.5 Hz, 1H), 5.64 (s, 2H), 4.49–4.34 (m, 3H), 2.14 (s, 3H), 2.10 (s, 3 H), 2.06 (s, 3 H); δ_C(75 MHz, $CDCl_3$) 170.4 (C), 170.0 (C), 169.5 (C), 153.3 (CH), 151.8 (C), 147.9 (C), 144.1 (C), 143.3 (CH), 135.1 (C), 132.9 (C), 130.4 (C), 129.6 (2 × CH), 129.5 (2 × CH), 126.8 (CH), 86.6 (CH), 80.6 (CH), 73.3 (CH), 70.7 (CH), 63.1 (CH_2), 53.8 (CH_2), 20.9 (CH_3), 20.6 (CH_3), 20.5 (CH_3); HRMS (ESI) calcd for $C_{25}H_{25}ClN_7O_7$ $[M+H]^+$: 570.1499 found 570.1499.

(2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(6-(1-(4-fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate 8d. δ_H(300 MHz, $CDCl_3$) 9.00 (s, 1H), 8.64 (s, 1H), 8.24 (s, 1H), 7.33–7.29 (m, 2H), 7.07–7.01 (m, 2H), 6.26 (d, $J = 5.3$ Hz, 1H), 5.95 (appt, $J = 5.5$ and 5.3 Hz, 1H), 5.67 (br dd, $J = 5.5$ and 4.5 Hz, 1H), 5.62 (s, 2H), 4.48–4.34 (m, 3H), 2.13 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H); δ_C(75 MHz, $CDCl_3$) 170.4 (C), 169.7 (C), 169.4 (C), 163.0 (d, $J = 248.7$ Hz, C), 153.2 (CH), 151.8 (C), 147.9 (C), 144.0 (C), 143.3 (CH), 130.3$_4$ (C), 130.3 (d, $J = 3.3$ Hz, C), 130.1 (d, $J = 8.2$ Hz, 2 × CH), 126.8 (CH), 116.3 (d, $J = 22.0$ Hz, 2 × CH), 86.5 (CH), 80.5 (CH), 73.2 (CH), 70.7 (CH), 63.1 (CH_2), 53.7 (CH_2), 20.8 (CH_3), 20.6 (CH_3), 20.4 (CH_3); HRMS (ESI) calcd for $C_{25}H_{25}FN_7O_7$ $[M+H]^+$: 554.1794 found 554.1793.

(2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(6-(1-(4-methylbenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate 8e. δ_H(300 MHz, $CDCl_3$) 9.01 (s, 1H), 8.62 (s, 1H), 8.24 (s, 1H), 7.23 (d, $J = 8.3$ Hz, 2H), 7.17 (d, $J = 8.3$ Hz, 2H), 6.26 (d, $J = 5.3$ Hz, 1H), 5.95 (t, $J = 5.5$ Hz, 1H), 5.68 (br dd, $J = 5.5$ and 4.5 Hz, 1H), 5.62 (s, 2H), 4.48–4.34 (m, 3H), 2.33 (s, 3H), 2.15 (s, 3H), 2.10 (s, 3H), 2.06 (s, 3H); δ_C(75 MHz, $CDCl_3$) 170.4 (C), 169.7 (C), 169.5 (C), 153.3 (CH), 151.8 (C), 148.1 (C), 144.0 (C), 143.1 (CH), 139.0 (C), 131.4 (C), 130.4 (C), 130.0 (2 × CH), 128.3 (2 × CH), 126.8 (CH), 86.5 (CH), 80.6 (CH), 73.3 (CH), 70.7 (CH), 63.1 (CH_2), 53.4 (CH_2), 21.3 (CH_3), 20.9 (CH_3), 20.6 (CH_3), 20.5 (CH_3); HRMS (ESI) calcd for $C_{26}H_{28}N_7O_7$ $[M+H]^+$: 550.2045 found 550.2044.

(2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(6-(1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate 8f. δ_H(300 MHz, $CDCl_3$) 9.00 (s, 1H), 8.60 (s, 1H), 8.24 (s, 1H), 7.28 (d, $J = 8.7$ Hz, 2H), 6.89 (d, $J = 8.7$ Hz, 2H), 6.26 (d, $J = 5.3$ Hz, 2H), 5.95 (appt, $J = 5.5$ and 5.3 Hz, 1H), 5.67 (br dd, $J = 5.5$ and 4.5 Hz, 1H), 5.59 (s, 2H), 4.48–4.34 (m, 3H), 3.78 (s, 3H), 2.14 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H); δ_C(75 MHz, $CDCl_3$) 170.4 (C), 169.7 (C), 169.5 (C), 160.1 (C), 155.6 (C), 153.2$_4$ (CH), 153.1$_9$ (C), 151.8 (C), 143.2 (CH), 129.8 (2 × CH), 126.6 (CH), 126.4 (2 × C), 114.7 (2 × CH), 86.5 (CH), 80.5 (CH), 73.2 (CH), 70.7 (CH), 63.1 (CH_2), 55.4 (CH_3), 54.1 (CH_2), 20.9 (CH_3), 20.6 (CH_3), 20.5 (CH_3); HRMS (ESI) calcd for $C_{26}H_{28}N_7O_8$ $[M+H]^+$: 566.1994 found 566.1993.

(2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(6-(1-(2-bromobenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate 8g. δ_H(300 MHz, $CDCl_3$) δ 9.02 (s, 1H), 8.74 (s, 1H), 8.26 (s, 1H), 7.62 (br d, $J = 7.9$ Hz, 1H), 7.33–7.19 (m, 3H), 6.27 (d, $J = 5.5$ Hz, 1H), 5.96 (t, $J = 5.5$ Hz, 1H), 5.80 (s, 2H), 5.68 (br dd, $J = 5.5$ and 4.4 Hz, 1H), 4.49–4.35 (m, 3H), 2.15 (s, 3H), 2.11 (s, 3 H), 2.06 (s, 3 H); δ_C(75 MHz, $CDCl_3$) 170.4 (C), 169.7 (C), 169.5 (C), 153.3 (CH), 151.9 (C), 148.0 (C), 144.0 (C), 143.3 (CH), 134.0 (C), 133.4 (CH), 130.6 (CH), 130.4 (C and CH), 128.4 (CH), 127.2 (CH), 123.6 (C), 86.5 (CH), 80.6 (CH), 73.2 (CH), 70.7 (CH), 63.1 (CH_2), 54.2 (CH_2), 20.9 (CH_3), 20.6 (CH_3), 20.5 (CH_3); HRMS (ESI) calcd for $C_{25}H_{25}BrN_7O_7$ $[M+H]^+$: 614.0993 found 614.0992.

(2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(6-(1-(3-chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate 8h. δ_H(300 MHz, $CDCl_3$) 9.00 (s, 1H), 8.68 (s, 1H), 8.25 (s, 1H), 7.30–7.28 (m, 3H), 7.19–7.16 (m, 1H), 6.25 (d, $J = 5.3$ Hz, 2H), 5.95 (appt, $J = 5.5$ and 5.3 Hz, 1H), 5.67 (br dd, $J = 5.5$ and 4.5 Hz, 1H), 5.63 (s, 2H), 4.47–4.33 (m, 3H), 2.13 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H); δ_C(75 MHz, $CDCl_3$) 170.4 (C), 169.7 (C), 169.5 (C), 153.2 (CH), 151.8 (C), 147.8 (C), 144.1 (C), 143.3 (CH), 136.4 (C), 135.1 (C), 130.6 (CH), 130.4 (C), 129.1 (CH), 128.2 (CH), 127.0 (CH), 126.2 (CH), 86.5 (CH), 80.5 (CH), 73.2 (CH), 70.7 (CH), 63.1 (CH_2), 53.7 (CH_2), 20.8 (CH_3), 20.6 (CH_3), 20.4 (CH_3); HRMS (ESI) calcd for $C_{25}H_{25}ClN_7O_7$ $[M+H]^+$: 570.1499 found 570.1499.

(2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(6-(1-(3,4-dichlorobenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate 8i. δ_H(300 MHz, $CDCl_3$) 9.00 (s, 1H), 8.69 (s, 1H), 8.25 (s, 1H), 7.43–7.40 (m, 2H), 7.14 (br dd, $J = 8.3$ and 2.3 Hz, 1H), 6.25 (d, $J = 5.3$ Hz, 1H), 5.95 (appt, $J = 5.5$ and 5.3 Hz, 1H), 5.67 (br dd, $J = 5.5$ and 4.7 Hz, 1H), 5.62 (s, 2H), 4.47–4.33 (m, 3H), 2.13 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H); δ_C(75 MHz, $CDCl_3$) 170.4 (C), 169.7 (C), 169.5 (C), 153.2 (CH), 151.8 (C), 147.7 (C), 144.2 (C), 143.4 (CH), 134.6 (C), 133.4 (C), 133.2 (C), 131.3 (CH), 130.4 (C), 130.0 (CH), 127.3 (CH), 126.9 (CH), 86.6 (CH), 80.5 (CH), 73.2 (CH), 70.7 (CH), 63.1 (CH_2), 53.1 (CH_2), 20.8 (CH_3), 20.6 (CH_3), 20.4 (CH_3); HRMS (ESI) calcd for $C_{25}H_{24}N_7O_7Cl_2$ $[M+H]^+$: 604.1109 found 604.1118.

(2*R*,3*R*,4*R*,5*R*)-2-(acetoxymethyl)-5-(6-(1-(4-nitrobenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate 8j. mp 142.5 °C; δ_H(300 MHz, $CDCl_3$) 9.03 (s, 1H), 8.75 (s, 1H), 8.26 (s, 1H), 8.22 (d, $J = 8.8$ Hz, 2H), 7.48 (d, $J = 8.8$ Hz, 2H), 6.26 (d, $J = 5.1$ Hz, 1H), 5.96 (appt, $J = 5.5$ and 5.3 Hz, 1H), 5.79 (s, 2H), 5.68 (br dd, $J = 5.3$ and 4.7 Hz, 1H), 4.49–4.34 (m, 3H), 2.15 (s, 3H), 2.10 (s, 3 H), 2.06 (s, 3 H); δ_C(75 MHz, $CDCl_3$) 170.4 (C), 169.7 (C), 169.5 (C), 153.3 (CH), 151.8 (C), 148.3 (C), 147.6 (C), 144.4 (C), 143.4 (CH), 141.4 (CH), 130.5 (C), 128.8 (2 × CH), 127.2 (CH), 124.5 (2 × CH), 112.9 (C), 86.7 (CH), 80.5 (CH), 73.3 (CH), 70.7 (CH), 63.1 (CH_2), 20.8 (CH_3), 20.6 (CH_3), 20.5 (CH_3); HRMS (ESI) calcd for $C_{25}H_{25}N_8O_9$ $[M+H]^+$: 581.1739 found 581.1743

8k. δ_H(300 MHz, $CDCl_3$) 9.02 (s, 2H), 8.86 (s, 2H), 8.27 (s, 2H), 6.28 (d, $J = 5.3$ Hz, 1H), 5.99 (appt, $J = 5.5$ and 5.3 Hz, 1H), 5.70 (br dd, $J = 5.3$ and 4.7 Hz, 1H), 4.62–4.58 (m, 4H), 4.50–4.36 (m, 8H), 2.15 (s, 3H), 2.12 (s, 3 H), 2.08 (s, 3 H); δ_C(75 MHz, $CDCl_3$) 170.5 (2 × C), 169.7 (2 × C), 169.5 (2 × C), 153.3 (2 × CH), 151.8 (2 × C), 147.8 (2 × C), 143.7 (2 × C), 143.4 (2 × CH), 130.5 (2 × C), 127.8 (2 × CH), 86.6 (2 × CH), 80.6 (2 × CH), 73.3 (2 × CH), 70.7 (2 × CH), 61.1 (2 × CH_2), 47.2 (2 × CH_2), 30.7 (CH_2), 20.9 (2 × CH_3), 20.7 (2 × CH_3), 20.5 (2 × CH_3); HRMS (ESI) calcd for $C_{39}H_{43}N_{14}O_{14}$ $[M+H]^+$: 931.3078 found 931.3080

(2*R*,3*S*,4*R*,5*R*)-2-(acetoxymethyl)-5-(6-(1-(3-azidopropyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diyl diacetate 8l. δ_H(300 MHz, $CDCl_3$) 9.04 (s, 1H), 8.77 (s, 1H), 8.28 (s, 1H), 6.28 (d, $J = 5.3$ Hz, 1H), 5.98 (appt, $J = 5.5$ and 5.3 Hz, 1H), 5.68 (br dd, $J = 5.5$ and 4.5 Hz, 1H), 4.60 (t, $J = 6.8$ Hz, 1H), 4.50–4.35 (m, 3H), 3.41 (t, $J = 6.3$ Hz, 2H), 2.31–2.22 (m, 2H), 2.15 (s, 3H), 2.12 (s, 3 H), 2.08 (s, 3 H); δ_C(75 MHz, $CDCl_3$) 170.4 (C), 169.7 (C), 169.5 (C), 153.2 (CH), 151.8 (C), 147.8 (C), 143.5 (C), 143.3 (CH), 130.4 (C), 127.4 (CH), 86.6 (CH), 80.6 (CH), 73.3 (CH), 70.7 (CH), 63.1 (CH_2), 48.1 (CH_2), 47.7 (CH_2), 29.6 (CH_2), 20.9 (CH_3), 20.7 (CH_3), 20.5 (CH_3); HRMS (ESI) calcd for $C_{21}H_{25}N_{10}O_7$ $[M+H]^+$: 529.1902 found 529.1907

General procedure for the synthesis of compounds 9a–9k

A solution of NH_3/MeOH (0.7 mL) was added over triacetate **9** (70 μmol). The mixture was stirred overnight at room temperature and followed by TLC. Once reaction was complete by TLC, the mixture was evaporated under reduced pressure and purified by column chromatography using dichloromethane–methanol (85/15) as eluent to afford the triazole derivatives **9a–9k**.

(2*R*,3*R*,4*S*,5*R*)-2-(6-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)-5-(hydroxymethyl)-tetrahydrofuran-3,4-diol 9a. mp 76–77 °C; δ_H(300 MHz, DMSO) 9.21 (s, 1H), 8.98 (s, 1H), 8.90 (s, 1H), 7.31–7.50 (m, 5H), 6.07 (d, $J = 5.5$ Hz, 1H), 5.79 (s, 2H), 5.61 (br s, 1H), 5.26 (br s, 1H), 5.15–5.12 (m, 1H), 4.64 (br s, 1H), 4.21 (br s, 1H), 4.99 (br s, 1H), 3.75–3.68 (m, 1H), 3.62–3.56 (m, 1H); δ_C(75 MHz, $CDCl_3$) 151.9 (CH), 150.8 (C), 146.8 (C), 145.3 (CH), 142.8 (C), 134.3 (C), 130.6 (C), 129.3 (2 × CH), 129.1 (CH), 128.2 (2 × CH), 127.4 (CH), 91.5 (CH), 87.7 (CH), 74.0 (CH), 72.6 (CH), 63.2 (CH_2), 54.6 (CH_2); HRMS (ESI) calcd for $C_{19}H_{20}N_7O_4$ $[M+H]^+$ 410.1571 found 410.1570; HPLC purity grade. 254 nm: 98.5%; 300 nm: 100%.

(2*R*,3*R*,4*S*,5*R*)-2-(6-(1-(4-bromobenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 9b. mp 126–127 °C; δ_H(300 MHz, DMSO) 9.24 (s, 1H), 8.98 (s, 1H), 8.92 (s, 1H), 7.60 (d, $J = 8.4$ Hz, 2H), 7.38 (d, $J = 8.4$ Hz, 2H), 6.08 (d, $J = 5.5$ Hz, 1H), 5.79 (s, 2H), 5.60 (br s, 1H), 5.29 (br s, 1H), 5.15 (br s, 1H), 4.66 (appt, $J = 5.3$ and 5.1 Hz, 1H), 4.22 (br t, $J = 4.3$ Hz, 1H), 4.00 (br q, $J = 3.8$ Hz, 1H), 3.73 (br dd, $J = 12.1$ and 3.4 Hz, 1H), 3.60 (br dd, $J = 12.1$ and 3.6 Hz, 1H); δ_C(75 MHz, DMSO) 152.2 (CH), 151.6 (C), 146.7 (C), 145.2 (CH), 142.4 (C), 135.3 (C), 131.8 (2 × CH), 130.4 (2 × CH), 129.7 (C), 128.4 (CH), 121.6 (C), 87.8 (CH), 85.7 (CH), 73.7 (CH), 70.2 (CH), 61.2 (CH_2), 52.3 (CH_2); HRMS (ESI) calcd for $C_{19}H_{19}N_7O_4Br$ $[M+H]^+$ 488.0676 found 488.0678; HPLC purity grade. 254 nm: 92%; 300 nm: 99%

(2*R*,3*R*,4*S*,5*R*)-2-(6-(1-(4-chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 9c. mp 118–119 °C; δ_H(300 MHz, DMSO) 9.24 (s, 1H), 8.98 (s, 1H), 8.91 (s, 1H), 7.53–7.39 (m, 4H), 6.08 (d, $J = 5.5$ Hz, 1H), 5.80 (s, 2H), 5.57 (br s, 1H), 5.25 (br s, 1H), 5.15–5.11 (br t, $J = 5.5$ Hz, 1H), 4.68–4.63 (m, 1H), 4.24–4.19 (m, 1H), 4.02–3.98 (q, $J = 3.8$ Hz, 1H), 3.77–3.68 (m, 1H), 3.63–3.56 (m, 1H); δ_C(75 MHz, DMSO) 152.2 (CH), 151.6 (C), 146.7 (C), 145.1 (CH), 142.4 (C), 134.9 (C), 133.0 (C), 130.1 (2 × CH), 129.7 (C), 128.9 (2 × CH), 128.4 (CH), 87.8 (CH), 85.7 (CH), 73.7 (CH), 70.2 (CH), 61.2 (CH_2), 52.2 (CH_2); HRMS (ESI) calcd for $C_{19}H_{19}N_7O_4Cl$ $[M+H]^+$ 444.1182 found 444.1181; HPLC purity grade. 254 nm: 96%; 300 nm: 99%

(2*R*,3*R*,4*S*,5*R*)-2-(6-(1-(4-fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 9d. mp 97–98 °C; δ_H(300 MHz, $CDCl_3$–MeOD = 1/1) 8.88 (s, 1H), 8.77 (s, 1H), 8.50 (s, 1H), 7.40–7.35 (m, 2H), 7.09–7.03 (m, 2H), 6.04 (d, $J = 6.2$ Hz, 1H), 5.65 (s, 2H), 4.80 (br dd, $J = 5.9$ and 5.5 Hz, 1H), 4.38 (dd, $J = 5.1$ and 2.7 Hz, 1H), 4.24–4.22 (m, 1H), 3.93 (dd, $J = 12.7$ and 2.3 Hz, 1H), 3.76 (dd, $J = 12.7$ and 2.3 Hz); δ_C(75 MHz, $CDCl_3$–MeOD = 1/1) 163.4 (d, $J = 248.2$ Hz, C), 152.3 (CH), 151.8 (C), 145.9 (CH), 143.7 (C), 130.8 (d, $J = 3.3$ Hz, C), 130.6 (d, $J = 8.3$ Hz, 2 × CH), 127.6 (CH) 116.5 (d, $J = 22.1$ Hz, 2 × CH), 90.8 (CH), 87.3 (CH), 74.7 (CH), 71.7 (CH), 62.7 (CH_2), 54.0 (CH_2); HRMS (ESI) calcd for $C_{19}H_{19}N_7O_4F$ $[M+H]^+$ 428.1477 found 428.1481; HPLC purity grade. 254 nm : 100%; 300 nm : 100%

(2*R*,3*S*,4*R*,5*R*)-2-(hydroxymethyl)-5-(6-(1-(4-methylbenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diol 9e. mp 90–91 °C; δ_H(300 MHz, DMSO) δ 9.17 (s, 1H), 8.97 (s, 1H), 8.90 (s, 1H), 7.32 (d, $J = 8.0$ Hz, 2H), 7.20 (d, $J = 8.0$ Hz, 2H), 6.07 (d, $J = 5.5$ Hz, 1H), 5.73 (s, 2H), 5.58 (br s, 1H), 5.27 (br s, 1H), 5.15–5.12 (m, 1H), 4.65 (br s, 1H), 4.21 (br s, 1H), 4.02–3.98 (m, 1H), 3.75–3.69 (m, 1H), 3.63–3.56 (m, 1H), 2.28 (s, 3H); δ_C(75 MHz, DMSO) 152.2 (CH), 151.6 (C), 146.8 (C), 145.1 (CH), 142.4 (C), 137.7 (C), 132.8 (C), 129.7 (C), 129.4 (2 × CH), 128.2 (2 × CH), 128.1 (CH), 87.8 (CH), 85.7 (CH), 73.7 (CH), 70.2 (CH), 61.2 (CH_2), 52.8 (CH_2), 20.7 (CH_3); HRMS (ESI) calcd for $C_{20}H_{22}N_7O_4$ $[M+H]^+$ 424.1728 found 424.1726; HPLC purity grade. 254 nm: 88%; 300 nm: 97%.

(2*R*,3*S*,4*R*,5*R*)-2-(hydroxymethyl)-5-(6-(1-(4-methoxybenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diol 9f. mp 167–168 °C; δ_H(300 MHz, DMSO) 9.15 (s, 1H), 8.97 (s, 1H),

8.90 (s, 1H), 7.40 (d, $J = 8.5$ Hz, 2H), 6.95 (d, $J = 8.5$ Hz, 2H), 6.07 (d, $J = 5.3$ Hz, 1H), 5.70 (s, 2H), 5.57 (br s, 1H), 5.26 (br s, 1H), 5.14 (br s, 1H), 4.65 (br s, 1H), 4.21 (br s, 1H), 3.99 (br s, 1H), 3.74–3.70 (m, 4H), 3.61–3.57 (m, 1H); δ_C(75 MHz, DMSO) 159.3 (C), 152.2 (CH), 151.6 (C), 146.8 (C), 145.1 (CH), 142.3 (C), 129.9 (2 × CH), 129.7 (C), 127.9 (CH), 127.7 (C), 114.2 (2 × CH), 87.8 (CH), 85.7 (CH), 73.7 (CH), 70.2 (CH), 61.2 (CH_2), 55.2 (CH_3), 52.6 (CH_2); HRMS (ESI) calcd for $C_{20}H_{22}N_7O_5$ $[M+H]^+$ 440.1677 found 440.1677; HPLC purity grade. 254 nm: 95%; 300 nm: 100%.

(2*R*,3*R*,4*S*,5*R*)-2-(6-(1-(2-bromobenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 9g. mp 80–81 °C; δ_H(300 MHz, DMSO) δ 9.17 (s, 1H), 8.99 (s, 1H), 8.90 (s, 1H), 7.72 (br d, $J = 7.6$ Hz, 1H), 7.47–7.42 (m, 1H), 7.37–7.29 (m, 1H), 6.07 (d, $J = 5.5$ Hz, 1H), 5.89 (s, 2H), 5.56 (br s, 1H), 5.25 (br s, 1H), 5.14–5.11 (m, 1H), 4.64 (br s, 1H), 4.22 (br s, 1H), 4.02–3.98 (m, 1H), 3.75–3.68 (m, 1H), 3.63–3.56 (m, 1H); δ_C(75 MHz, $CDCl_3$) 151.9 (CH), 150.8 (C), 146.7 (C), 145.5 (CH), 142.8 (C), 133.7 (C), 133.4 (CH), 130.7 (CH), 130.6 (C), 130.4 (CH), 128.4 (CH), 127.8 (CH), 91.4 (CH), 87.7 (CH), 74.0 (CH), 72.5 (CH), 63.1 (CH_2), 54.3 (CH_2); HRMS (ESI) calcd for $C_{19}H_{19}N_7O_4Br$ $[M+H]^+$ 488.0676 found 488.0676; HPLC purity grade. 254 nm: 93%; 300 nm: 96%

(2*R*,3*R*,4*S*,5*R*)-2-(6-(1-(3-chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)-*H*-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 9h. mp 67–68 °C; δ_H(300 MHz, DMSO) 9.28 (s, 1H), 8.98 (s, 1H), 8.92 (s, 1H), 7.52 (s, 1H), 7.45–7.56 (m, 3H), 6.08 (br d, $J = 5.3$ Hz, 1H), 5.82 (br s, 2H), 5.58 (br s, 1H), 5.26 (br s, 1H), 5.16–5.13 (m, 1H), 4.68–4.63 (m, 1H), 4.22 (br s, 1H), 4.00 (br s, 1H), 3.76–3.69 (m, 1H), 3.64–3.55 (m, 1H); δ_C(75 MHz, DMSO) 152.2 (CH), 151.6 (C), 146.7 (C), 145.2 (CH), 142.4 (C), 138.2 (C), 133.3 (C), 130.8 (CH), 129.7 (C), 128.5 (CH), 128.3 (CH), 128.1 (CH), 126.9 (CH), 87.8 (CH), 85.7 (CH), 73.7 (CH), 70.2 (CH), 61.2 (CH_2), 52.2 (CH_2); HRMS (ESI) calcd for $C_{19}H_{19}N_7O_4Cl$ $[M+H]^+$ 444.1182 found 444.1183; HPLC purity grade. 254 nm: 96%; 300 nm: 99%.

(2*R*,3*R*,4*S*,5*R*)-2-(6-(1-(3,4-dichlorobenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)-5-(hydroxymethyl)tetrahydrofuran-3,4-diol 9i. mp 117–118 °C; δ_H(300 MHz, DMSO) 9.29 (s, 1H), 8.98 (s, 1H), 8.92 (s, 1H), 7.75 (d, $J = 2.1$ Hz, 1H), 7.68 (d, $J = 8.3$ Hz, 1H), 7.40 (dd, $J = 8.3$ and 2.1 Hz, 1H), 6.08 (d, $J = 5.5$ Hz, 1H), 5.81 (s, 2H), 5.57 (br s, 1H), 5.26 (br s, 1H), 5.15–5.12 (m, 1H), 4.65 (br s, 1H), 4.21 (br s, 1H), 4.02–3.98 (m, 1H), 3.76–3.69 (m, 1H), 3.63–3.56 (m, 1H); δ_C(75 MHz, DMSO) 152.2 (CH), 151.6 (C), 146.7 (C), 145.2 (CH), 142 5 (C), 136.8 (C), 131.3 (C), 131.1 (CH), 131.0$_9$ (C), 130.3 (CH), 129.7 (C), 128.6 (CH), 128.5 (CH), 87.8 (CH), 85.7 (CH), 73.7 (CH), 70.2 (CH), 61.2 (CH_2), 51.6 (CH_2); HRMS (ESI) calcd for $C_{19}H_{18}N_7O_4Cl_2$ $[M+H]^+$ 478.0792 found 478.0789; HPLC purity grade. 254 nm: 96%; 300 nm: 99%.

(2*R*,3*S*,4*R*,5*R*)-2-(hydroxymethyl)-5-(6-(1-(4-nitrobenzyl)-1*H*-1,2,3-triazol-4-yl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diol 9j. δ_H(300 MHz, DMSO) δ 9.33 (s, 1H), 8.99 (s, 1H), 8.92 (s, 1H), 8.26 (d, $J = 8.8$ Hz, 2H), 7.63 (d, $J = 8.8$ Hz, 2H), 6.08 (d, $J = 5.5$ Hz, 1H), 5.99 (s, 2H), 5.57 (br d, $J = 5.7$ Hz, 1H), 5.25 (br d, $J = 4.7$ Hz, 1H), 5.13 (br t, $J = 5.4$ Hz, 1H), 4.68–4.63 (m, 1H), 4.24–4.19 (m, 1H), 4.02–3.98 (m, 1H), 3.76–3.69 (m, 1H), 3.63–3.56 (m, 1H); δ_C(75 MHz, DMSO) δ 152.2 (CH), 151.6 (C), 147.3 (C), 146.6 (C), 145.2 (CH), 143.3 (C), 142.5 (C), 129.7 (C), 129.2 (2 × CH), 128.8 (CH), 124.0 (2 × CH), 87.8 (CH), 85.7 (CH), 73.71 (CH), 70.2 (CH), 61.2 (CH_2), 52.1 (CH_2); HRMS (ESI) calcd for $C_{19}H_{19}N_8O_6$ $[M+H]^+$ 455.1422 found 455.1424; HPLC purity grade: 254 nm: 98%; 300 nm: 100%

Drugs, reagents and cell culture medium

RPMI 1640, glucose, fetal calf serum (10%), geneticin (G418), penicillin, streptomycin (100 IU mL^{-1}), DMEM/Nut mix F12, Glutamax were purchased from Invitrogen (Cergy Pontoise, France). Isobutyl methyl xanthine (IBMX), 8 cyclopentyl-1,3-dipropylxanthine (DPCPX), and methanol were from SIGMA Aldrich (St Quentin Fallavier, France). Chem 3 growth supplement was from Millipore (Billerica MA USA). [H^3] DPCPX were from Amersham (Orsay, France).

Cell culture

Stable cell line human recombinant A_1AR, was obtained from Millipore (CHO, CHEM 3 cells; Millipore, Billerica MA, USA). Cells were cultured (5% CO_2, 37 °C) in RPMI 1640, containing 4.5 g L^{-1} of glucose, supplemented with 10% of fetal calf serum, 0.8 mg mL^{-1} of geneticin (G418), 100 IU mL^{-1} of penicillin and streptomycin and diluted (1/100) with Chem 3 growth supplement. Cells were cultured (5% CO_2, 37 °C) in DMEM/Nut mix F12 with fetal calf serum and glutamax, according to the recommendations of the manufacturer.

cAMP concentration determination

Cells were incubated (2×10^5 per well) 30 min with 1 mM of IBMX, and increasing concentration of tested drugs. Total amount of cAMP was determined by immunoassay (cAMP Biotrak Enzyme immunoasay, EIA system, Amersham Pharmacia Biotrak Orsay, France). EC 50 is the concentration of drug causing half maximal inhibition. Data obtained from the dose–response curved were analyzed by non linear regression analysis (GraphPad Prism, Software, San Diego USA).

Binding assay

Frozen CHO cells were subjected to three freeze-thaw cycles (–80 °C, +20 °C), centrifuged (4 °C, 5000 g, 20 min), suspended in 4.5 mL of binding buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.4), and homogenized just prior to the binding assay. We used [^{3}H] DPCPX selective A1 adenosine receptors (A1ARs) ligand. Homogenates of CHO cell membranes (200 µl for a total volume of 250 µl) were incubated (90 min at room temperature) with [^{3}H] DPCPX at the concentration of 5nM (10X KD) and in the presence of increasing concentration of drugs tested (10^{-8} to 10^{-4} M), to obtained displacement curves. Binding experiments were performed in triplicate. Bound and free radioactivities were separated by vacuum-filtration of the sample through Whatman (Breenford, UK) GF/C glass-fiber filters. Cold binding buffer (1 mL) was added to the sample before filtering. The filter was washed three times and bound radioactivity was determined with a Beckman LS-1800 liquid scintillation spectrometer. A weighted non-linear least-square curve fitting program, Graph Pad Prism (Graph Pad Software Inc., San Diego, CA, USA) was used for the computer analysis. Non-specific binding was defined as binding in

the presence of 10 μM of unlabeled ligand. EC 50 was defined as the concentration of drug that induce the half maximal effects.

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