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Original article

Design, synthesis and structure–activity relationships of a series of 9-substituted adenine derivatives as selective phosphodiesterase type-4 inhibitors

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Abstract

Adenine derivatives substituted in position 9 have been demonstrated to have potent cyclic nucleotide phosphodiesterase (PDE) inhibition properties with high selectivity toward PDE-4. Starting from our initial lead compound 9-(2-fluorobenzyl)-N^6-methyl-2-trifluoromethyladenine (**4**, NCS613), we designed and synthesized a new series of 9-substituted derivatives for developing structure–activity relationship studies. This new series of derivatives showed increased potencies and better selectivity profiles. Structural modifications were achieved in parallel on three different positions of the adenine ring, and led to the following observations: (i) introduction of a lipophilic substituent such as trifluoromethyl, *n*-propyl group or iodine in the C-2 position is favourable for both the PDE-4 inhibitory activity and the selectivity towards other isoenzymes; (ii) functionalization of the N9 benzyl group with a 2-methoxy substituent led to remarkably more active compounds; (iii) replacement of the N^6-methylamino moiety by other amino groups is detrimental to the activity. Among all derivatives prepared, the 9-(2-methoxybenzyl)-N^6-methyl-2-trifluoromethyladenine (**9r**), 9-(2-methoxybenzyl)-N^6-methyl-2-*n*-propyladenine (**9s**), and the 2-iodo-9-(2-methoxybenzyl)-N^6-methyladenine (**13b**) were found to be the most potent inhibitors within this series (PDE-4-IC_{50} = 1.4, 7.0, and 0.096 nM, respectively). Compared to our reference compound **4**, which showed an IC_{50} of 42 nM, the derivative **13b** was found 450-fold more potent. Moreover, 2-iodo-9-(2-methoxybenzyl)-N^6-methyladenine (**13b**) and 9-(2-methoxybenzyl)-N^6-methyl-2-trifluoromethyladenine (**9r**), were at least 50 000–150 000 times more selective for the PDE-4 than for the other PDE families. Additionally, these new derivatives showed improved efficiency in inhibiting the TNFα release from mononuclear cells from healthy subjects (e.g. adenines **7l**, **9s** and **13b**). Thus, compounds **7l**, **9r**, **9s** and **13b** are among the most potent and selective PDE-4 inhibitors reported so far and represent very promising pharmacological tools for a better understanding of the signal transduction involving cyclic AMP within the cell: this pathway is implicated in the physiology and the pathophysiology of inflammation, asthma and autoimmune disorders.

Keywords: Adenine; Phosphodiesterase; Type-4; TNFα; Inflammation

1. Introduction

Cyclic nucleotide phosphodiesterases (PDEs), responsible for the hydrolysis of key second messengers cyclic AMP (cAMP) and cyclic GMP (cGMP), have been classified into at least 11 major families with respect to their substrate specificity, sequence similarity, and

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sensitivity to inhibitory drugs [1,2]. PDE-4 is a cAMP-specific enzyme, separated in four human isoforms (PDE-4 A, B, C, and D) [3], and represents a promising potential molecular target for the development of new antiasthmatic and antiinflammatory drugs. Furthermore, PDE-4 inhibitors have demonstrated efficacy in models of dermatitis, rheumatoid arthritis, multiple sclerosis, autoimmune diseases and various gastrointestinal and neurological diseases [1,4–8]. Thus, several PDE-4 inhibitors are under clinical development. Unfortunately, the development of PDE-4 inhibitors, such as rolipram (**1**, Fig. 1) and structurally-related compounds has been so far limited by various side effects, such as nausea, emesis, gastric acid secretion, or central nervous system activation [7,8,11,12]. Thus, the design of novel, potent and selective second generation of PDE-4 inhibitors with reduced side effects represent a critical need and is still a challenge in the pharmaceutical industry [5,8–10]. One way to increase both the potency and the selectivity of PDE-4 inhibitors, and thus improve the therapeutic index, is to develop new compounds featuring original chemical structures, different than those of the known PDE-4 inhibitors [13], if the side effects are not mechanism-based.

Several years ago, Kelley and Sokoro described the 9-(2-fluorobenzyl)-N^6-methyladenine (**3**) as a potent anticonvulsant [14]. We preliminarily demonstrated that this compound presents potent anxiolytic and sedative properties [15], and was a relatively potent PDE-4 inhibitor ($IC_{50} = 2.0$ μM) [16]. Furthermore, initial structure–activity relationship (SAR) studies around 9-substituted adenine derivatives allowed us to identify 9-(2-fluorobenzyl)-N^6-methyl-2-trifluoromethyladenine (**4**), as a potent PDE-4 inhibitor ($IC_{50} = 0.042$ μM), with a high selectivity vs PDE-3 [16]. Moreover, compound **4** elicited anti-inflammatory properties [17], and marked dose-dependent inhibition of arachidonate release from human mononuclear cells stimulated with *N*-formyl-Met–Leu–Phe, a suitable model to investigate the in vitro anti-inflammatory activity of PDE-4 inhibitors [18]. In addition, compound **4** and several 9-substituted adenine derivatives elicited a concentration-dependent inhibition of the TNFα release from mononuclear cells stimulated with lipopolysaccharide (LPS) [18]. Moreover, the fact that this series of PDE-4 inhibitors did not stimulate the in vivo gastric acid secretion in rats suggests that they may produce fewer gastrointestinal side effects than other PDE-4 inhibitors, such as compound **2** (Fig. 1) [17].

The aim of this work was to optimize our selective PDE-4 lead inhibitor 9-(2-fluorobenzyl)-N^6-methyl-2-trifluoromethyladenine (**4**, Fig. 1). For this purpose a systematic exploration of possible substitutions and functionalizations on three different positions on the adenine ring (positions 2, 6, and 9) was undertaken.

2. Chemistry

Three main synthetic approaches leading to adenine derivatives are described in the literature, depending upon the position of the substitution to be explored: (i) syntheses started from a substituted pyrimidine nucleus followed by imidazole ring construction (exploration of position 8) [14]; (ii) or starting from the corresponding imidazole ring, then construction of the pyrimidine nucleus (exploration of position 2) [19–22]; (iii) or direct substitution of the preformed purine ring (exploration of N-9 substitution) [16,23–27].

We previously demonstrated that within this series of adenine PDE-4 inhibitors, no substitution is tolerated at position 7. Thus, both alkylation of the purine ring and

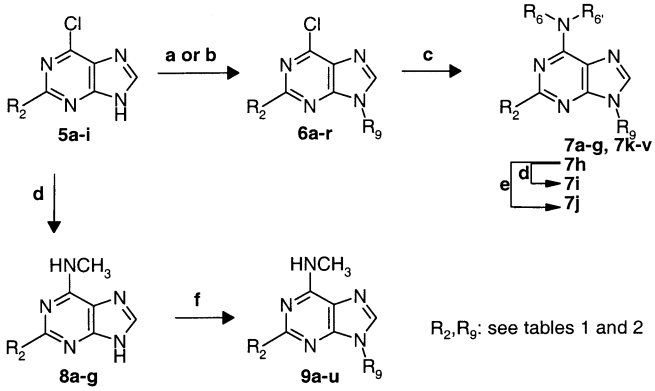


Fig. 2. Access to the adenine derivatives by chloropurines and adenines alkylation. Reagents: (a) R_9Cl, NaH; (b) PhCH(OH)COOMe, PPh_3, DEAD; (c) $HNR_6R_{6'}$; (d) H_2NCH_3; (e) $NaBH_4$; (f) R_9Cl, K_2CO_3, TBAI.

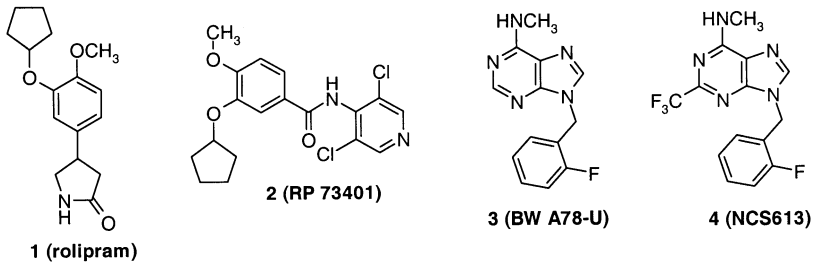


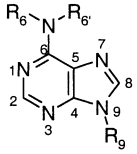
Fig. 1. Structures of PDE type-4 inhibitors.

cyclization of an appropriate 4-aminoimidazole-5-carboxamide were used to further explore substitutions and functionalizations around positions 2 and 9 [16].

A classical route to prepare the 9-substituted adenines **7** and **9** deals with direct alkylation of the purine ring using different experimental conditions [16,23–27]. Thus, different N^9-alkyl adenine derivatives **7** were readily prepared by alkylation of corresponding chloropurines **5** [25] with the appropriate alkyl halides and sodium hydride in dimethylformamide (Fig. 2) [16,24]. Under these conditions, alkylation occurred mainly at position N9 (70–85%), and the undesired N7 isomer (15–30%) could be easily removed by silica gel column chromatography. Subsequent treatment of compounds **6** with various amines provided the desired adenines **7** (Table 1) [24]. In the case of compound **7h**, the Mitsunobu procedure was used to afford the desired product in 73% overall yield [26].

More recently, direct alkylation of adenines **8** under phase-transfer catalyst conditions was reported to constitute a powerful synthetic tool for the preparation of N9 alkyl adenines [27]. This procedure was superior to the previously described synthesis using the classical alkylation of chloropurines, because it was assumed to lead to regioselective alkylation at position 9 of the purine ring (Fig. 2). However, in our hands, while the N9-alkylated derivatives were obtained as major products, traces of N7-substituted compounds were also

Table 1
Physical constants and inhibition of PDE-4 by 6,9-disubstituted adenines



Compound	Salt	m.p. (°C)	Yield [a] (%)	Elemental analysis [b]	R_6	$R_{6'}$	R_9	PDE-4-IC50 [c] (μM) or (%) of inhibition [d]
7a	di-HCl	224	79	$C_{12}H_{12}N_6 \cdot 2HCl$	NH_2	H	Bn	30
7b	HCl	234	83	$C_{12}H_{11}N_5O \cdot HCl \cdot 1H_2O$	OH	H	Bn	32%
7c	HCl	248	84	$C_{16}H_{17}N_5 \cdot HCl \cdot 1H_2O$			Bn	21
7d	di-HCl	> 300	53	$C_{16}H_{18}N_6 \cdot 2HCl$			Bn	107
7e	free base	221	64	$C_{13}H_{13}N_5O$	OMe	H	Bn	90
7f	HCl	248	78	$C_{13}H_{12}ClN_5 \cdot HCl \cdot 0.7H_2O$	Me	H	3-ClBn	4.0
7g	HCl	266	72	$C_{14}H_{13}N_5O_2 \cdot HCl$	Me	H	2,3-(methylenedioxy)Bn	0.89
7h	HCl	204	73	$C_{15}H_{15}N_5O_2 \cdot HCl \cdot 1.6H_2O$	Me	H	(MeOCO)(Ph)CH	12
7i	HCl	182	68	$C_{15}H_{16}N_6O \cdot HCl \cdot 2.1H_2O$	Me	H	(MeNHCO)(Ph)CH	91
7j	HCl	220	80	$C_{14}H_{15}N_5O \cdot HCl$	Me	H	$(HOCH_2)(Ph)CH$	19
7k	HCl	258	73	$C_{12}H_{11}N_5 \cdot HCl \cdot 0.2H_2O$	Me	H	Ph	10
9a	HCl	200	76	$C_{13}H_{13}N_5 \cdot HCl \cdot 0.5H_2O$	Me	H	Bn	4.6
9b	HCl	210	51	$C_{10}H_{15}N_5 \cdot HCl \cdot 3H_2O$	Me	H	*n*-Bu	13
9c	HCl	230	57	$C_{11}H_{15}N_5 \cdot HCl \cdot 2.3H_2O$	Me	H	c-Pen	2.9
9d	HCl	252	20	$C_{11}H_{17}N_5 \cdot HCl \cdot 1.5H_2O$	Me	H	neopenthyle	6.6
9e	HCl	212	40	$C_{18}H_{31}N_5 \cdot HCl$	Me	H	dodecyle	61%
9f	HCl	232	30	$C_{14}H_{15}N_5 \cdot HCl \cdot 0.2H_2O$	Me	H	$Ph(CH_2)_2$	22
9g	HCl	205	46	$C_{15}H_{17}N_5 \cdot HCl \cdot 1.5H_2O$	Me	H	$Ph(CH_2)_3$	4.8
9h	HCl	274	37	$C_{13}H_{12}ClN_5 \cdot HCl \cdot 0.4H_2O$	Me	H	4-ClBn	14
9i	HCl	287	38	$C_{13}H_{12}BrN_5 \cdot HCl$	Me	H	2-BrBn	1.0
9j	HCl	257	25	$C_{14}H_{15}N_5O \cdot HCl \cdot 0.2H_2O$	Me	H	2-(MeO)Bn	0.50
9k	HCl	222	20	$C_{14}H_{15}N_5O \cdot HCl \cdot 0.4H_2O$	Me	H	4-(MeO)Bn	8.8
9l	HCl	205	56	$C_{14}H_{13}N_5O \cdot HCl \cdot 0.5H_2O$	Me	H	$PhCOCH_2$	27
9m	HCl	215	40	$C_{21}H_{21}N_5 \cdot HCl$	Me	H	$(Ph)_2CH(CH)_2$	11
9n	HCl	244	73	$C_{20}H_{17}N_5O \cdot HCl$	Me	H	4-(PhCO)$PhCH_2$	13

[a] For the final step.
[b] C, H, N.
[c] The IC_{50} was calculated by linear regression (correlation coefficient $r = 0.95$) and represents the mean value of three determinations. The experimental error is about 15%.
[d] At 100 μM of final drug concentration.

observed. Despite the fact that the separation of these polar adenine derivatives led to poor overall yields, this synthetic method appeared to be particularly powerful for an expeditious preparation of N9-substituted derivatives in one-step procedure. Yields and physical constants of adenine derivatives are listed in Table 1 and Table 2.

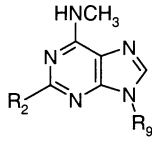
The presence of an amino moiety at position 2 constitutes another versatile synthetic route to access at 2,9-disubstituted adenine derivatives. As shown in Fig. 3, alkylation of the 2-amino-6-chloropurine (**10**) gave 9-substituted intermediates **11a** and **11b** in a regioselective manner [27]. The amino group was converted to the key iodo intermediates (**12a, b**) [28], which were subsequently displaced with methylamine to afford adenines **13a** and **13b**.

The last step of the synthesis involved palladium-catalyzed cross-coupling reactions and led to 2-substituted derivatives **14a–c** (Fig. 3) [29,30]. Finally, the iodine in position 2 could be easily displaced by sodium methanethiolate leading to **14d**.

3. Pharmacology, results and discussion

All N9-substituted adenine derivatives were tested for their inhibitory capacities of bovine PDE-4 as described in the Section 5. The concentration that inhibited 50% of the enzymatic activity (IC_{50}), or the percentage of inhibition at a final concentration of 100 μM of each drug were determined at 1 μM cAMP concentration. Data are listed in Table 1 and Table 2. Isoenzyme selectivity profile of the most potent inhibitors was obtained by comparing the IC_{50} values of the compounds against PDE-4 with their inhibitory activity against PDE-1, -2, -3, and -5 (Table 3). Inhibition of TNFα release from mononuclear cells stimulated with LPS was also reported for the compounds **7l**, **9s** and **13b**,

Table 2
Physical constants and inhibition of PDE-4 by 2,9-disubstituted N^6-methyladenines



Compound	Salt	m.p. (°C)	Yield [a] (%)	Elemental analysis [b]	R_2	R_9	PDE-4-IC_{50} [c] (μM)
4, [16]	free base	138	–	–	CF_3	2-FBn	0.042
7l	HCl	186	75	$C_{16}H_{19}N_5 \cdot HCl$	*n*-Pr	Bn	0.088
7m	HCl	181	73	$C_{16}H_{19}N_5 \cdot HCl$	*i*-Pr	Bn	0.12
7n	HCl	238	81	$C_{19}H_{23}N_5 \cdot HCl$	c-Hex	Bn	0.16
7o	HCl	236	85	$C_{17}H_{21}N_5 \cdot HCl$	*t*-Bu	Bn	7.1
7p	HCl	276	60	$C_{21}H_{19}N_5 \cdot HCl$	PhCH=CH	Bn	9.0
7q	di-HCl	236	73	$C_{13}H_{14}N_6 \cdot 2HCl$	NH_2	Bn	6.9
7r	free base	180	62	$C_{14}H_{13}F_3N_6O$	CF_3	2-(MeO)-3-pyridylCH_2	0.10
7s	HCl	236	82	$C_{15}H_{17}N_5O \cdot HCl$	Me	2-(MeO)Bn	0.061
7t	HCl	242	84	$C_{15}H_{17}N_5O \cdot HCl$	Me	4-(MeO)Bn	0.92
7u	HCl	218	80	$C_{16}H_{19}N_5O \cdot HCl$	Me	2-(MeO)Ph$(CH_2)_2$	54
7v	HCl	98	83	$C_{12}H_{19}N_5O_2 \cdot HCl \cdot 1.5H_2O$	Me	$CH_3O(CH_2)_2O(CH_2)_2$	20
9o	HCl	188	62	$C_{18}H_{23}N_5 \cdot HCl$	*n*-Pen	Bn	1.8
9p	HCl	230	67	$C_{21}H_{21}N_5 \cdot HCl \cdot 2H_2O$	Ph$(CH_2)_2$	Bn	2.2
9q	HCl	174	65	$C_{22}H_{23}N_5 \cdot HCl \cdot 1.3H_2O$	Ph$(CH_2)_3$	Bn	1.3
9r	Free base	169	45	$C_{15}H_{14}F_3N_5O \cdot 0.2H_2O$	CF_3	2-(MeO)Bn	0.0014
9s	HCl	119	79	$C_{17}H_{21}N_5O \cdot HCl \cdot 0.5H_2O$	*n*-Pr	2-(MeO)Bn	0.0070
9t	HCl	241	74	$C_{23}H_{23}N_5O \cdot HCl \cdot 0.5H_2O$	*n*-Pr	4-(PhCO)PhCH_2	0.92
9u, [17]	$MeSO_3H$	146	–	–	Me	2-FBn	0.19
13a	HCl	221	63	$C_{13}H_{12}IN_5 \cdot HCl$	I	Bn	0.030
13b	HCl	192	78	$C_{14}H_{14}IN_5O \cdot HCl$	I	2-(MeO)Bn	0.000096
14a	HCl	195	70	$C_{16}H_{15}N_5 \cdot HCl$	$CH_3C{\equiv}C$	Bn	0.092
14b	HCl	151	60	$C_{17}H_{17}N_5O \cdot HCl \cdot 1.6H_2O$	$CH_3C{\equiv}C$	2-(MeO)Bn	0.015
14c	HCl	205	72	$C_{17}H_{19}N_5O \cdot HCl$	CH_3CH=CH	2-(MeO)Bn	0.055
14d	HCl	158	88	$C_{15}H_{17}N_5OS \cdot HCl$	CH_3S	2-(MeO)Bn	0.080

[a] For the final step.
[b] C, H, N.
[c] The IC_{50} was calculated by linear regression (correlation coefficient $r = 0.95$) and represents the mean value of three determinations. The experimental error is about 15%.

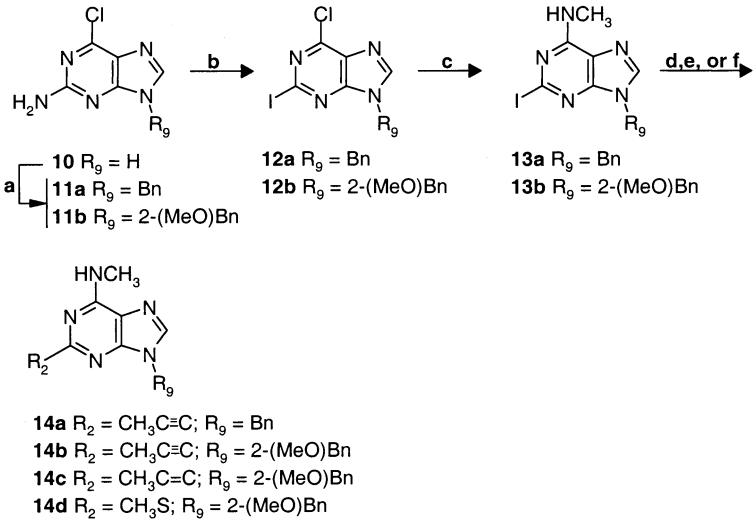


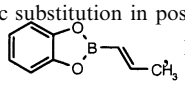
Fig. 3. Access to the adenine derivatives by palladium cross-coupling reactions or aromatic nucleophilic substitution in position 2. Reagents: (a) R_9Cl, K_2CO_3, TBAI; (b) isoamyl nitrite, CH_2I_2; (c) H_2NCH_3; (d) $CH_3C{\equiv}CH$, CuI, $PdCl_2$, PPh_3, TEA; (e)  $Pd(PPh_3)_4$, NaOH; (f) MeSNa.

Table 3
Selectivity of adenine derivatives for PDE-4 vs PDE-1, -2, -3 and -5

Compound	R_2	R_9	IC_{50} [a] (μM) or (%) of inhibition [b]				
			PDE-1	PDE-2	PDE-3	PDE-4	PDE-5
4 (NCS613)	CF_3	2-FBn	39	41%	380	0.045	5
7l (NCS675)	*n*-Pr	Bn	33%	16%	43%	0.082	24
9r (NCS728)	CF_3	2-(MeO)Bn	20%	16%	24%	0.0014	74
9s (NCS700)	*n*-Pr	2-(MeO)Bn	62	15	54%	0.0070	41
13b (NCS706)	I	2-(MeO)Bn	49	26%	33%	0.000096	15

[a] The IC_{50} was calculated by linear regression (correlation coefficient $r = 0.95$) and represents the mean value of three determinations. The experimental error is about 15%.
[b] At 100 μM of final drug concentration.

which were found as three of the most potent PDE-4 inhibitors within this series.

The various substitutions and functionalizations on the three different positions (2, 6 and 9) were designed to complete the initial SAR studies, which already led to the identification of the potent PDE-4 inhibitor 9-(2-fluorobenzyl)-N^6-methyl-2-trifluoromethyladenine (**4**, $IC_{50} = 45$ nM) [16]. Improving PDE-4 activity and selectivity vs PDE-1, -2, -3 and -5 was also one of the goods of the present study.

As reported in our previous works [16], a single substitution with a methyl group on the exocyclic nitrogen atom at position 6 of the adenine ring was optimal. In particular, both *N,N*-dimethyl and *N,N*-unsubstituted derivatives were 50- to 300-fold less active than the *N*-monomethyl adenine **4**. SAR studies carried out with the new series of adenine derivatives confirmed these preliminary results. As shown in Table 1, replacement of the methylamino group at position 6 with a pyrrolidyl or a piperazinyl moiety induced dramatic reductions in PDE-4 inhibitory activity (compare compounds **7c** and **7d** with **9a**). Other substitutions with an amino, hydroxy or methoxy group were detrimental to the activity (compare **7a**, **7b** and **7e** with **9a**).

Furthermore, a series of homologues of the 9-benzyl derivative **9a** was first tested to optimize the linker between the adenine ring and the phenyl moiety at position 9. Thus, a methylene group was found to be a better spacer than an ethylene one (compare **9a** and **9f**). When the linker is absent (9-phenyl substituent in **7k**) [31], the activity was 2-fold diminished. Surprisingly, the phenylpropyl derivative **9g** was as active as the reference

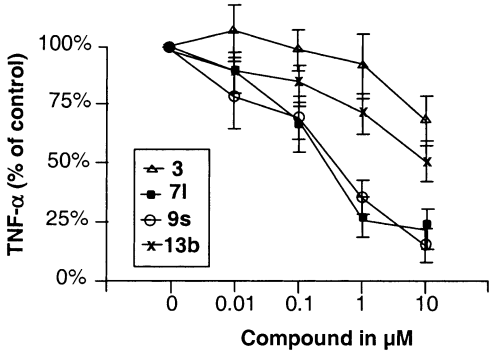


Fig. 4. Effects of compound **3**, **7l**, **9s** and **13b** on TNFα release by LPS-activated peripheral blood mononuclear cells. The properties of compounds **3**, **7l**, **9s** and **13b** on TNFα production by mononuclear cells was evaluated by ELISA as described in Experimental Section in presence of 5 μg mL^{-1} of LPS. Results (mean ± S.E. mean) express TNFα production as the percentage of LPS activated control.

compound **9a**. Except the 9-cyclopentyl derivative (**9c**), all other lipophilic, non-aromatic substituents at the same position led to less active compounds (see **9b**, **9d** and **9e**).

Different substituents were introduced on the aromatic carbocycle of derivative **9a**. As shown in Table 1, introduction of a substituent in *ortho* position of the phenyl ring appeared to be especially beneficial. Thus, the 2-bromobenzyl and 2-methoxybenzyl groups at position 9 produced compounds 5- to 10-fold more potent (compare **9i** and **9j** with **9a**). Other 9-benzyladenines functionalized in positions 3 (**7f**), 4 (**9h**, **9k**, **9n**) or α (**7h**–**j**) of the benzyl ring and other N9-functionalized products (**7g**, **9l**, **9m**) were either less active than the unsubstituted benzyl derivative **9a** or equipotent (**7f**). The introduction of other methoxy-substituted side chains led to less active compounds when compared to the 2-methoxybenzyl **7s** (see cpds **7u**, **7v**, Table 2).

Earlier SAR studies around position 2 established that the introduction of a methyl or a trifluoromethyl group strongly increase the potency of the resulting compounds [16]. In a similar manner, when compared to **9a** ($IC_{50} = 4.6$ μM), the *n*-propyl (**7l**) and propynyl derivatives (**14a**) were found more potent ($IC_{50} \approx 90$ nM), indicative of some degree of steric tolerance at position 2 (Table 2). However, replacing the *n*-propyl or propynyl groups by more bulky alkyl substituents (**7m**–**o**, **9o**) or phenylalkyl groups (**7p**, **9p** and **9q**) always produced less active compounds. Surprisingly, introducing an iodine atom in position 2 led to the most potent compound within the 9-benzyl series (IC_{50} **13a** = 30 nM), whereas the 2-amino derivative **7q** was less active than the unsubstituted compound **9a**.

Finally, combining beneficial substituent effects in both positions 2 and 9 led to new compounds with increased potencies. Thus, synergistic effects were observed when the 9-(2-methoxybenzyl) moiety was introduced in derivatives with the most appropriate substituent in position 2. In each case the 2-methoxy derivative was found more active (3 to 300-fold) than the corresponding unsubstituted benzyl or 2-fluorobenzyl compounds (compare **9u** and **7s**, **4** and **9r**, **7l** and **9s**, **13a** and **13b** or **14a** and **14b**). In particular, the 2-*n*-propyl (**9s**) and 2-trifluoromethyl (**9r**) derivatives exhibited high potencies with IC_{50} values equal to 7.0 and 1.4 nM, respectively. However, the iodo analogue **13b** was found to be the most potent PDE-4 inhibitor with subnanomolar PDE-4 IC_{50} (0.096 nM). Moreover, these structural optimizations around the positions 2 and 9 provided improved PDE-4 selectivity profiles vs PDE-1, -2, -3, and -5 (Table 3). Thus, considering simultaneously inhibition of these five different isoenzymes (PDE-1–5), compounds **9r** and **13b** were at least 50 000–150 000 times more selective toward the PDE-4.

Finally, we examined the effects of compound **7l**, **9s** and **13b** on TNFα release by LPS-activated peripheral blood mononuclear cells from healthy donors (Fig. 4). These compounds elicited a strong concentration-dependent inhibition of the TNFα release and were much more potent (**7l**-$IC_{50} = 0.5$ μM, **9s**-$IC_{50} = 0.7$ μM and **13b**-$IC_{50} = 9$ μM, respectively) than the reference compound **3** ($IC_{50} > 10$ μM) [17]. Unexpectedly, we found no direct correlation between the activity of the compounds **3**, **7l**, **9s** and **13b** in PDE-4 inhibition and TNFα inhibition. Such a finding suggests that accumulation of cAMP is a major way to decrease TNFα secretion but certainly not the only one.

4. Conclusions

In summary, structural optimization within the 9-substituted adenine series of PDE-4 inhibitors, led to compounds with very potent PDE-4 inhibitory activities (IC_{50} values in the nanomolar range) and PDE-4 selectivity vs PDE-1, -2, -3, and -5. In particular, the 9-(2-methoxybenzyl)-N^6-methyl-2-*n*-propyladenine (**9s**) and 9-(2-methoxybenzyl)-N^6-methyl-2-trifluoromethyladenine (**9r**) derivatives showed high potencies with IC_{50} values of 7.0 and 1.4 nM, respectively. Surprisingly, the iodo derivative **13b** was found to be the most potent PDE-4 inhibitor with subnanomolar PDE-4 IC_{50} value ($IC_{50} = 0.096$ nM), 450-fold more active than our initial lead compound 9-(2-fluorobenzyl)-N^6-methyl-2-trifluoromethyladenine (**4**). Moreover, these structural optimizations combining both positions 2 and 9 provided improved PDE-4 selectivity vs PDE-1, -2, -3, and -5 with an index of selectivity reaching 50 000–150 000 for compounds **9r** and **13b**. Thus, compounds **9r**, **9s** and **13b** appear as three of the most potent and selective PDE-4 inhibitors so far known. Additionally, these new derivatives showed improved efficacies in inhibiting the TNFα release from mononuclear cells from healthy subjects, as shown with compounds **7l**, **9s**, and **13b**.

This family of adenine derivatives that may compete with cAMP within its binding site, differs from other dialkoxyaryl-related compounds, and other known PDE-4 inhibitors. This work thus highlights new potent and selective PDE-4 inhibitors (e.g. compounds **9r**, **9s** and **13b**) as very promising pharmacological tools for a better understanding of the signal transduction (including cAMP) within the cell.

5. Experimental section

5.1. Chemical synthesis

5.1.1. General

Reagents used for the synthesis were purchased from Sigma-Aldrich (Isle d'Abeau Chesnes, France) and Lancaster (Bischheim-Strasbourg, France). [^{3}H]-cAMP (1.1–1.85 TBq nmol^{-1}, TRK 498) was obtained from Amsterdam (Les Ulis, France). With the exception of THF and Et_2O, all solvents were obtained from commercial suppliers and used without further purification. These two solvents were freshly distilled from sodium benzophenone ketyl. Flash chromatography was performed on Geduran® Silica gel Si 60 (40–63 μm, Merck). Thin-layer chromatography was carried out using plates Silica gel 60 F_{254} (Merck). The spots were visualized either under UV light ($\lambda = 254$ nm) or by spraying with molybdate reagent (H_2O–concentrated H_2SO_4–$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$–$(NH_4)_2Ce(SO_4)_4\cdot 2H_2O$, 90/10/25/1, v/v/w/w) and charring at 140 °C for a few minutes. All chemical yields are unoptimized and generally represent the result of a single experiment.

^{1}H-NMR were recorded on a Bruker AC 200 (200 MHz) or a Bruker DPX 300 (300 MHz) spectrophotometer at room temperature. Chemical shifts are given in ppm (δ), coupling constants (J) are in hertz (Hz) and signals are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; quint., quintuplet; m, multiplet; br s, broad singlet.

The mass spectra were obtained on a Mariner API-TOF.

Melting points were determined with a Mettler FP62 apparatus and are uncorrected. Elemental analyses were performed by the CNRS department of microanalysis (CNRS, Vernaison, France) and are indicated only by the elemental symbols within ±0.4% of the theoretical values unless otherwise noted.

5.1.2. 6-Chloro-2-methylpurine hydrochloride (**5b**)

To a solution of 5-aminoimidazole-4-carboxamide hydrochloride (500 mg, 3.08 mmol), 4-(*N,N*-dimethylamino)pyridine (12.5 mg, 0.10 mmol) in anhydrous pyridine (25 mL) was slowly added acetyl chloride (241 μl, 3.39 mmol). The mixture was stirred at 80 °C for 8 h, concentrated under reduced pressure, then diluted with cold water (20 mL). The precipitate was collected, washed with water and suspended in potassium hydrogen carbonate (0.5 N, 140 mL). The resulting suspension was heated to reflux for 4 h, concentrated under reduced pressure, cooled to 0 °C, and adjusted to pH 4 with 10% aqueous HCl. The solid was collected, washed with water and EtOH, and dried in a vacuum oven at 50 °C for 1 h. Then, phosphorus oxychloride (10 mL, 107 mmol) and *N,N*-dimethylaniline (2 mL, 15.8 mmol) were added and the resultant mixture was heated to reflux under nitrogen for 1 h, cooled down to room temperature, concentrated under reduced pressure and diluted with CH_2Cl_2. This solution was treated with HCl gas at 0 °C. The precipitate solid was collected, washed with CH_2Cl_2 and ether to give **5b** as a white solid which was identical in all respects with the product reported in Ref. [16].

5.1.3. 6-Chloro-2-n-propylpurine hydrochloride (**5c**)

The title compound was prepared (70%) from 5-aminoimidazole-4-carboxamide hydrochloride and butyryl chloride as described for **5b**: ^{1}H-NMR (200 MHz, CD_3OD) δ 1.00 (t, $J = 7.3$, 3H, CH_3), 1.81–1.95 (m, 2H, CH_2), 3.03 (t, $J = 7.5$, 2H, CH_2), 9.46 (s, 1H, 8-H); *m/z* 197 $(M+H)^+$.

5.1.4. 6-Chloro-2-isopropylpurine hydrochloride (**5d**)

The title compound was prepared (63%) from 5-aminoimidazole-4-carboxamide hydrochloride and 2-methylpropionyl chloride as described for **5b**: ^{1}H-NMR (200 MHz, CD_3OD) δ 1.39 (d, $J = 6.9$, 6H, $2CH_3$), 2.96 (m, 1H, CH), 9.38 (s, 1H, 8-H); *m/z* 197 $(M+H)^+$.

5.1.5. 6-Chloro-2-cyclohexylpurine hydrochloride (**5e**)

The title compound was prepared (73%) from 5-aminoimidazole-4-carboxamide hydrochloride and cyclohexanecarboxylyl chloride as described for **5b**: ^{1}H-NMR (200 MHz, CD_3OD) δ 1.25–2.09 (m, 10H, $5CH_2$), 2.92–3.08 (m, 1H, CH), 9.39 (s, 1H, 8-H); *m/z* 237 $(M+H)^+$.

5.1.6. 6-Chloro-2-tert-butylpurine hydrochloride (**5f**)

The title compound was prepared (66%) from 5-aminoimidazole-4-carboxamide hydrochloride and pivaloyl chloride as described for **5b**: ^{1}H-NMR (200 MHz, CD_3OD) δ 1.48 (s, 9H, $3CH_3$), 9.40 (s, 1H, 8-H); *m/z* 211 $(M+H)^+$.

5.1.7. 6-Chloro-2-trans-styrylpurine hydrochloride (**5g**)

The title compound was prepared (80%) from 5-aminoimidazole-4-carboxamide hydrochloride and *trans*-cinnamoyl chloride as described for **5b**: ^{1}H-NMR (200 MHz, CD_3OD) δ 7.46 (d, $J = 16.1$, 1H, CH), 7.36–7.71 (m, 5H, 5CH), 7.85 (d, $J = 16.1$, 1H, CH), 9.45 (s, 1H, 8-H); *m/z* 257 $(M+H)^+$.

*5.1.8. 6-Chloro-2-n-pentylpurine hydrochloride (**5h**)*

The title compound was prepared (78%) from 5-aminoimidazole-4-carboxamide hydrochloride and caproyl chloride as described for **5b**: ^{1}H-NMR (200 MHz, CD_3OD) δ 0.90 (t, $J = 7.0$, 3H, CH_3), 1.30–1.42 (m, 4H, $2CH_2$), 1.80–1.95 (m, 2H, CH_2), 3.05 (t, $J = 7.6$, 2H, CH_2), 9.48 (s, 1H, 8-H); *m/z* 225 $(M+H)^+$.

*5.1.9. 6-Chloro-2-(3-phenylpropyl)purine hydrochloride (**5i**)*

The title compound was prepared (71%) from 5-aminoimidazole-4-carboxamide hydrochloride and 4-phenylbutyryl chloride as described for **5b**: ^{1}H-NMR (200 MHz, CD_3OD) δ 2.13–2.29 (m, 2H, CH_2), 2.71 (t, $J = 7.7$, 2H, CH_2), 3.02–3.11 (m, 2H, CH_2), 7.08–7.26 (m, 5H, 5CH), 9.45 (s, 1H, 8-H); *m/z* 295 $(M+Na)^+$.

*5.1.10. General procedure for the alkylation of 6-chloropurines (**5**) and subsequent displacement using various amines provided adenines **7a–j**, **7l–p** and **7r–v** (method A)*

To a stirring solution of 6-chloropurine hydrochloride **5** (0.976 mmol) in dry DMF (4 mL) was added at 0 °C NaH (50% in mineral oil, 121 mg). After 5 min, the reaction mixture was allowed warm up to room temperature: the requisite alkyl halide (2.61 mmol) was then added and stirring was continued for 24 h. The mixture was diluted with water (15 mL), extracted with ethyl acetate (60 mL), dried (Na_2SO_4), and concentrated to dryness under reduced pressure. Chromatography on silica (AcOEt) afforded the desired N9-alkylated product **6**. Sequential addition of ethanol (10 mL) and the appropriate amine (5 mmol) was carried out under argon atmosphere. The resulting solution was stirred for 24 h at room temperature. After evaporation of the solvent, the residue was diluted with ethyl acetate (60 mL), washed with cold water (20 mL) and saturated sodium bicarbonate (10 mL), dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography on silica (AcOEt–CH_2Cl_2–EtOH, 50:40:10) afforded adenines **7** as colorless solids, which were eventually converted into the HCl salt. An analytically pure sample was obtained by recrystallization from ethanol and diethyl ether. Unoptimized percentage yields, referring to the last step, are given for each product (Tables 1 and 2).

*5.1.11. 9-Benzyl-6-hydrazinopurine di hydrochloride (**7a**)*

The title compound was prepared from 6-chloropurine, benzyl chloride and hydrazine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, DMSO-d_6) δ 5.62 (s, 2H, CH_2), 7.40–7.49 (m, 5H, 5CH), 8.60 (s, 1H, 2-H), 8.77 (s, 1H, 8-H), 10.21 (br s, 3H, $NH+NH_2$). Anal. Found: $C_{12}H_{12}N_6 \cdot 2HCl$ (C, H, N).

*5.1.12. 9-Benzyl-6-hydroxylaminopurine hydrochloride (**7b**)*

The title compound was prepared from 6-chloropurine, benzyl chloride and hydroxylamine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, DMSO-d_6+CD_3OD) δ 5.62 (s, 2H, CH_2), 7.42–7.49 (m, 5H, 5CH), 8.51 (s, 1H, 2-H), 8,80 (s, 1H, 8-H). Anal. Found: $C_{12}H_{11}N_5O \cdot HCl \cdot 1H_2O$ (C, H, N).

*5.1.13. 9-Benzyl-6-(1-pyrrolidyl)purine hydrochloride (**7c**)*

The title compound was prepared from 6-chloropurine, benzyl chloride and pyrrolidine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, DMSO-d_6) δ 2.13–2.20 (m, 4H, $2CH_2$), 3.86–3.92 (m, 2H, CH_2), 4.30–4.36 (m, 2H, CH_2), 5.65 (s, 2H, CH_2), 7.41–7.49 (m, 5H, 5CH), 8.58 (s, 1H, 2-H), 8.80 (s, 1H, 8-H). Anal. Found: $C_{16}H_{17}N_5 \cdot HCl \cdot 1H_2O$ (C, H, N).

*5.1.14. 9-Benzyl-6-(1-piperazinyl)purine di hydrochloride (**7d**)*

The title compound was prepared from 6-chloropurine, benzyl chloride and piperazine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.34–3.39 (m, 4H, $2CH_2$), 4.55–4.63 (m, 4H, $2CH_2$), 5.56 (s, 2H, CH_2), 7.39–7.48 (m, 5H, 5CH), 8.48 (s, 1H, 2-H), 8.58 (s, 1H, 8-H), 9.57 (br s, 1H, NH). Anal. Found: $C_{16}H_{18}N_6 \cdot 2HCl$ (C, H, N).

*5.1.15. 9-Benzyl-N^6-methoxyadenine (**7e**)*

The title compound was prepared from 6-chloropurine, benzyl chloride and methoxylamine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, $CDCl_3$+CD_3OD) δ 3.97 (s, 3H, CH_3), 5.35 (s, 2H, CH_2), 7.25–7.37 (m, 5H, 5CH), 7.74 (s, 1H, 2-H), 8.17 (s, 1H, 8-H). Anal. Found: $C_{13}H_{13}N_5O$ (C, H, N).

*5.1.16. 9-(3-Chlorobenzyl)-N^6-methyladenine hydrochloride (**7f**)*

The title compound was prepared from 6-chloropurine, 3-chlorobenzyl chloride and methylamine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.13 (br s, 3H, CH_3), 5.53 (s, 2H, CH_2), 7.32–7.47 (m, 4H, 4CH), 8.50 (s, 1H, 2-H), 8.66 (s, 1H, 8-H), 9.75 (br s, 1H, NH). Anal. Found: $C_{13}H_{12}ClN_5 \cdot HCl \cdot 0.7H_2O$ (C, H, N).

*5.1.17. N^6-methyl-9-(2,3-methylenedioxybenzyl)adenine hydrochloride (**7g**)*

The title compound was prepared from 6-chloropurine, 2,3-methylenedioxybenzyl chloride and methyla-

mine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.10 (br s, 3H, CH_3), 5.45 (s, 2H, CH_2), 6.03 (s, 2H, CH_2), 6.71–6.91 (m, 3H, 3CH), 8.46 (s, 1H, 2-H), 8.51 (s, 1H, 8-H), 9.72 (br s, 1H, NH). Anal. Found: $C_{14}H_{13}N_5O_2 \cdot HCl$ (C, H, N).

5.1.18. Methyl R,S-(±)-2-(6-methylaminopurin-9-yl)-2-phenylacetate hydrochloride (7h)

To a solution containing chloropurine (1.00 g, 6.45 mmol), methyl mandelate (2.15 g, 12.9 mmol), and triphenyl phosphine (2.55 g, 9.70 mmol) in THF (100 mL) was slowly added a solution of diethyl azodicarboxylate (1.53 mL, 9.70 mmol) in THF (50 mL) under argon atmosphere. The mixture was stirred at room temperature for 12 h. Volatiles were removed, and the residue was directly purified by chromatography (CH_2Cl_2–hexane–Et_2O, 60:30:10) to give compound **6d** (1.27 g, 65%) as a colorless syrup: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.85 (s, 3H, CH_3), 6.57 (s, 1H, CH), 7.39–7.53 (m, 5H, 5CH), 8.23 (s, 1H, 8-H), 8.76 (s, 1H, 2-H); *m/z* 303 $(M+H)^+$. A stirring solution of **6d** (1.00 g, 3.30 mmol) in EtOH (5 mL) was treated with methylamine (1.0 M in THF, 3.47 mL) at room temperature for 5 h. After evaporation of the solvent, the residue was chromatographed on silica and eluted (AcOEt–CH_2Cl_2–EtOH, 4:5:1), to afford a solid. Recrystallization from ethanol and diethyl ether gave **7h** (804 mg, 73%) as a colorless solid: ^{1}H-NMR (200 MHz, $CDCl_3$) δ: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 3.62 (s, 3H, CH_3), 3.91 (s, 3H, CH_3), 6.48 (s, 1H, CH), 7.38–7.50 (m, 5H, 5CH), 8.06 (s, 1H, 8-H), 8.22 (s, 1H, 2-H), 9.53 (br s, 1H, NH). Anal. Found: $C_{15}H_{15}N_5O_2 \cdot HCl \cdot 1.6H_2O$ (C, H, N).

5.1.19. R,S-(±)-N-methyl-2-(6-methylaminopurin-9-yl)-2-phenyl acetamide hydrochloride (7i)

Prepared from **6d** (500 mg, 1.65 mmol) and methylamine (1.0 M in THF, 8.00 mL) using the procedure described for compound **7h**. Compound **7i** (379 mg, 69%) was obtained as a colorless solid: ^{1}H-NMR (200 MHz, CD_3OD) δ 2.80 (s, 3H, CH_3), 3.20 (s, 3H, CH_3), 6.53 (s, 1H, CH), 7.42–7.56 (m, 5H, 5CH), 8.21 (s, 1H, 8-H), 8.42 (s, 1H, 2-H). Anal. Found: $C_{15}H_{16}N_6O \cdot HCl \cdot 2.1H_2O$ (C, H, N).

5.1.20. R,S-(±)-2-(6-methylaminopurin-9-yl)-2-phenylethanol hydrochloride (7j)

To a solution of methyl *R,S*-(±)-2-(6-methylaminopurin-9-yl)-2-phenylacetate hydrochloride (**7h**) (207 mg, 0.70 mmol) in anhydrous THF (5 mL) was added sodium borohydride (130 mg, 3.45 mmol) at 0 °C under argon atmosphere. The reaction mixture was allowed to warm up to room temperature and refluxed for 1 h. After cooling, volatiles were removed, and the residue was diluted with ethyl acetate, washed with water, dried (Na_2SO_4), and concentrated to dryness under reduced pressure. Chromatography on silica and elution (AcOEt–EtOH, 80:20) afforded the desired product as a colorless solid, which was converted to the HCl salt **7j**. Recrystallization from ethanol and diethyl ether yielded compound **7j** (171 mg, 80%) as analytically pure sample: ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.20 (br s, 3H, CH_3), 3.63 (s, 1H, OH), 4.17–4.67 (m, 2H, CH_2), 5.78–5.99 (m, 1H, CH), 7.32–7.42 (m, 5H, 5CH), 8.35 (s, 1H, 2-H), 8.58 (s, 1H, 8-H). Anal. Found: $C_{14}H_{15}N_5O \cdot HCl$ (C, H, N).

5.1.21. N^6-methyl-9-phenyladenine hydrochloride (7k)

The title compound was prepared following the literature procedure [36]: ^{1}H-NMR (200MHz, DMSO-d_6) δ 3.21 (s, 3H, CH_3), 7.63–8.00 (m, 5H, 5CH), 8.56 (s, 1H, 2-H), 8.89 (s, 1H, 8-H), 9.20 (br s, 1H, NH). Anal. Found: $C_{12}H_{11}N_5 \cdot HCl \cdot 0.2H_2O$ (C, H, N).

5.1.22. 9-Benzyl-N^6-methyl-2-n-propyladenine hydrochloride (7l)

The title compound was prepared from **5c**, benzyl chloride and methylamine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.03 (t, $J = 7.3$, 3H, CH_3), 1.85–2.04 (m, 2H, CH_2), 2.94 (t, $J = 7{,}5$, 2H, CH_2), 3.59 (d, $J = 5.5$, 3H, CH_3), 5.36 (s, 2H, CH_2), 7.28–7.40 (m, 5H, 5CH), 7.84 (s, 1H, 8-H), 9.44 (br s, 1H, NH). Anal. Found: $C_{16}H_{19}N_5 \cdot HCl$ (C, H, N).

5.1.23. 9-Benzyl-2-isopropyl-N^6-methyladenine hydrochloride (7m)

The title compound was prepared from **5d**, benzyl chloride and methylamine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.46 (d, $J = 6.9$, 6H, $2CH_3$), 3.21–3.24 (m, 1H, CH), 3.57 (d, $J = 5.5$, 3H, CH_3), 5.37 (s, 2H, CH_2), 7.29–7.45 (m, 5H, 5CH), 7.86 (s, 1H, 8-H), 9.60 (br s, 1H, NH). Anal. Found: $C_{16}H_{19}N_5 \cdot HCl$ (C, H, N).

5.1.24. 9-Benzyl-2-cyclohexyl-N^6-methyladenine hydrochloride (7n)

The title compound was prepared from **5e**, benzyl chloride and methylamine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.37–2.10 (m, 10H, $5CH_2$), 2.88–2.94 (m, 1H, CH), 3.59 (d, $J = 5.1$, 3H, CH_3), 5.36 (s, 2H, CH_2), 7.29–7.41 (m, 5H, 5CH), 7.83 (s, 1H, 8-H), 9.67 (br s, 1 H, NH). Anal. Found: $C_{19}H_{23}N_5 \cdot HCl$ (C, H, N).

5.1.25. 9-Benzyl-2-tert-butyl-N^6-methyladenine hydrochloride (7o)

The title compound was prepared from **5f**, benzyl chloride and methylamine following the general proce-

dure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.55 (s, 9H, $3CH_3$), 3.57 (d, $J = 5.5$, 3H, CH_3), 5.36 (s, 2H, CH_2), 7.27–7.40 (m, 5H, 5CH), 7.88 (s, 1H, 8-H), 10.34 (br s, 1 H, NH). Anal. Found: $C_{17}H_{21}N_5 \cdot HCl$ (C, H, N).

5.1.26. 9-Benzyl-N^6-methyl-2-trans-styryladenine hydrochloride (7p)

The title compound was prepared from **5g**, benzyl chloride and methylamine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.33 (d, $J = 5.1$, 3H, CH_3), 5.17 (s, 2H, CH_2), 7.08–7.13 (m, 8H, 8CH), 7.10–7.78 (m, 4H, 4CH), 7.81 (s, 1H, 8-H), 9.10 (br s, 1H, NH). Anal. Found: $C_{21}H_{19}N_5 \cdot HCl$ (C, H, N).

5.1.27. 2-Amino-9-benzyl-N^6-methyladenine di hydrochloride (7q)

A solution of **11a** (200 mg, 0.77 mmol) and methylamine (1.0 M in THF, 5 mL) in absolute ethanol (5 mL) was stirred for 24 h at room temperature. After evaporation of the solvent, the residue was chromatographed on silica (AcOEt–CH_2Cl_2–EtOH, 4:5:1), then recrystallized from ethanol and ether to give **7q** which was converted to the HCl salt (184 mg, 73%): ^{1}H-NMR (200 MHz, CD_3OD) δ 3.21 (s, 3H, CH_3), 5.38 (s, 2H, CH_2), 7.34–7.40 (m, 5H, 5CH), 8.52 (s, 1H, 8-H). Anal. Found: $C_{13}H_{14}N_6 \cdot 2HCl$ (C, H, N).

5.1.28. -9[(2-Methoxy-3-pyridyl)methyl]-N^6-methyl-2-trifluoromethyladenine (7r)

The title compound was prepared from 6-chloro-2-trifluoromethyladenine **5h** [16], and methylamine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.25 (br s, 3H, CH_3), 4.03 (s, 3H, CH_3), 5.36 (s, 2H, CH_2), 5.90 (br s, 1H, NH), 6.87–6.91 (m, 1H, CH), 7.64–7.67 (m, 1H, CH), 7.96 (s, 1H, 8-H), 8.14–8.16 (m, 1H, CH). Anal. Found: $C_{14}H_{13}F_3N_6O$ (C, H, N).

5.1.29. 9-(2-Methoxybenzyl)-2,N^6-dimethyladenine hydrochloride (7s)

The title compound was prepared from **5b** [16], 2-methoxybenzyl chloride and methylamine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.73 (s, 3H, CH_3), 3.59 (d, $J = 4.9$, 3H, CH_3), 3.88 (s, 3H, CH_3), 5.34 (s, 2H, CH_2), 6.91–7.33 (m, 4H, 4CH), 7.97 (s, 1H, 8-H), 9.19 (br s, 1H, NH). Anal. Found: $C_{15}H_{17}N_5O \cdot HCl$ (C, H, N).

5.1.30. 9-(4-Methoxybenzyl)-2,N^6-dimethyladenine hydrochloride (7t)

The title compound was prepared from **5b** [16], 4-methoxybenzyl chloride and methylamine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.74 (s, 3H, CH_3), 3.60 (d, $J = 4.9$, 3H, CH_3), 3.82 (s, 3H, CH_3), 5.30 (s, 2H, CH_2), 6.95–7.25 (m, 4H, 4CH), 7.82 (s, 1H, 8-H), 9.26 (br s, 1H, NH). Anal. Found: $C_{15}H_{17}N_5O \cdot HCl$ (C, H, N).

5.1.31. 9-(2-Methoxyphenethyl)-2,N^6-dimethyladenine hydrochloride (7u)

The title compound was prepared from **5b** [16], 2-methoxyphenethyl chloride and methylamine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.67 (s, 3H, CH_3), 3.17 (t, $J = 6.6$, 2H, CH_2), 3.59 (d, $J = 4.8$, 3H, CH_3), 3.75 (s, 3H, CH_3), 4.48 (t, $J = 6.6$, 2H, CH_2), 6.85–7.24 (m, 4H, 4CH), 7.48 (s, 1H, 8-H), 9.20 (br s, 1H, NH). Anal. Found: $C_{16}H_{19}N_5O \cdot HCl$ (C, H, N).

5.1.32. 9-[2-(2-Methoxyethoxy)ethyl]-2,N^6-dimethyladenine hydrochloride (7v)

The title compound was prepared from **5b** [16], 2-(2-methoxyethoxy)ethyl chloride and methylamine following the general procedure for the alkylation of 6-chloropurines (method A): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.69 (s, 3H, CH_3), 3.38 (s, 3H, CH_3), 3.49–6.68 (m, 7H, $2CH_2 + CH_3$), 3.82 (t, $J = 4.8$, 2H, CH_2), 4.41 (t, $J = 4.8$, 2H, CH_2), 8.10 (s, 1H, 8-H), 9.21 (br s, 1H, NH). Anal. Found: $C_{12}H_{19}N_5O_2 \cdot HCl \cdot 1.5H_2O$ (C, H, N).

5.1.33. N^6-methyladenine (8a)

A solution of 6-chloropurine (**5a**) (263 mg, 1.70 mmol) and methylamine (1.0 M in THF, 5 mL) in absolute ethanol (5 mL) was heated at 120 °C in a sealed tube for 2 h. After evaporation of the solvent, the residue was diluted with cold water (5 mL) then, the precipitate was filtered, sequentially washed with cold water, ethanol and Et_2O, then recrystallized from EtOH to yield **5a** (246 mg, 97%) as a white powder. This sample was identical in all respects with the product reported in Ref. [32].

5.1.34. N^6-methyl-2-n-pentyladenine (8b)

The title compound was prepared from **5h** following the procedure described for **8a**: ^{1}H-NMR (200 MHz, CD_3OD) δ 0.90 (t, $J = 6.6$, 3H, CH_3), 1.31–1.39 (m, 4H, $2CH_2$), 1.72–1.83 (m, 2H, CH_2), 2.74 (t, $J = 7.5$, 2H, CH_2), 3.13 (s, 3H, CH_3), 7.96 (s, 1H, 8-H); m/z 220 $(M+H)^+$.

5.1.35. N^6-methyl-2-trans-styryladenine (8c)

The title compound was prepared from **5g** following the procedure described for **8a**: ^{1}H-NMR (200 MHz,

DMSO-d_6) δ 3.20 (s, 3H, CH_3), 7.29–7.70 (m, 7H, 7CH), 8.00 (s, 1H, 8-H); *m/z* 274 $(M+Na)^+$.

*5.1.36. N^6-methyl-2-phenethyladenine (**8d**)*

A mixture of **8c** (210 mg, 0.83 mmol) and 10% Pd–C (30 mg) in absolute methanol (100 mL) was shaken in a hydrogenation apparatus at room temperature for 15 h. The catalyst was removed by filtration, washed with methanol and the filtrate was concentrated to dryness to give compound **8d** (187 mg, 88%) as a colorless solid: ^{1}H-NMR (200 MHz, CD_3OD) δ 2.98–3.11 (m, 4H, $2CH_2$), 3.14 (s, 3H, CH_3), 7.10–7.22 (m, 5H, 5CH), 7.96 (s, 1H, 8-H); *m/z* 276 $(M+Na)^+$.

*5.1.37. N^6-methyl-2-(3-phenylpropyl)adenine (**8e**)*

The title compound was prepared (89%) from **5i** following the procedure described for **8a**: ^{1}H-NMR (200 MHz, CD_3OD) δ 2.03–2.21 (m, 2H, CH_2), 2.71 (t, $J=7.3$, 2H, CH_2), 2.78 (t, $J=7.4$, 2H, CH_2), 3.13 (s, 3H, CH_3), 7.05–7.27 (m, 5H, 5CH), 7.95 (s, 1H, 8-H); *m/z* 290 $(M+Na)^+$.

*5.1.38. N^6-methyl-2-trifluoromethyladenine (**8f**)*

The title compound was prepared (95%) from 6-chloro-2-trifluoromethyladenine **5h** [16], following the procedure described for **8a**. A colorless solid which was identical in all respects with the product reported in the literature was obtained [33].

*5.1.39. N^6-methyl-2-n-propyladenine (**8g**)*

The title compound was prepared from **5c** following the procedure described for **8a**: ^{1}H-NMR (200 MHz, DMSO-d_6) δ 0.97 (t, $J=7.3$, 3H, CH_3), 1.72–1.91 (m, 2H, CH_2), 2.72 (t, $J=7.7$, 2H, CH_2), 3.13 (s, 3H, CH_3), 7.95 (s, 1H, 8-H); *m/z* 192 $(M+H)^+$.

*5.1.40. General procedure for the alkylation of N^6-methyladenines (**8**) provided adenines **9a–t** (method B)*

To a solution of N^6-methyladenine **8** (11.8 mmol), K_2CO_3 (1.63 g, 11.8 mmol), and tetra-*n*-butylammonium iodide (200 mg, 0.54 mmol) in dry DMF (16.0 mL) was added dropwise the requisite alkyl chloride (13 mmol). The resulting suspension was stirred for 24 h at room temperature. After evaporation of the solvent, the residue was diluted with cold water (50 mL) and extracted three times with ethyl acetate (60 mL). Organic layers were subsequently dried (Na_2SO_4) and concentrated to dryness under reduced pressure. Chromatography on silica and elution (AcOEt–EtOH, 9:1) afforded compounds **9** as a colorless solid.

*5.1.41. 9-Benzyl-N^6-methyladenine hydrochloride (**9a**)*

The title compound was prepared from **8a** and benzyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, CD_3OD) δ 3.20 (s, 3H, CH_3), 5.51 (s, 2H, CH_2), 7.30–7.39 (m, 5H, 5CH), 8.38 (s, 1H, 2-H), 8.40 (s, 1H, 8-H). Anal. Found: $C_{13}H_{13}N_5\cdot HCl\cdot 0.5H_2O$ (C, H, N).

*5.1.42. 9-n-Butyl-N^6-methyladenine hydrochloride (**9b**)*

The title compound was prepared from **8a** and *n*-butyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, CD_3OD) δ 1.00 (t, $J=7.3$, 3H, CH_3), 1.31–1.49 (m, 2H, CH_2), 1.86–1.97 (m, 2H, CH_2), 3.24 (s, 3H, CH_3), 4.37 (t, $J=7.1$, 2H, CH_2), 8.39 (s, 1H, 2-H), 8.44 (s, 1H, 8-H). Anal. Found: $C_{10}H_{15}N_5\cdot HCl\cdot 0.3H_2O$ (C, H, N).

*5.1.43. 9-Cyclopentyl-N^6-methyladenine hydrochloride (**9c**)*

The title compound was prepared from **8a** and benzyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, CD_3OD) δ 1.78–2.40 (m, 8H, $4CH_2$), 3.22 (s, 3H, CH_3), 8.40 (s, 1H, 2-H), 8.44 (s, 1H, 8-H). Anal. Found: $C_{11}H_{15}N_5\cdot HCl\cdot 2.3H_2O$ (C, H, N).

*5.1.44. N^6-methyl-9-neopentyladenine hydrochloride (**9d**)*

The title compound was prepared from **8a** and neopentyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, CD_3OD) δ 0.90 (s, 9H, $3CH_3$), 3.11 (s, 3H, CH_3), 4.04 (s, 2H, CH_2), 8.19 (s, 1H, 2-H), 8.28 (s, 1H, 8-H). Anal. Found: $C_{11}H_{17}N_5\cdot HCl\cdot 1.5H_2O$ (C, H, N).

*5.1.45. 9-Dodecyl-N^6-methyladenine hydrochloride (**9e**)*

The title compound was prepared from **8a** and dodecyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, CD_3OD) δ 0.89 (t, $J=6.4$, 3H, CH_3), 1.23–1.34 (m, 18H, $9CH_2$), 1.82 (m, 2H, CH_2), 3.20 (s, 3H, CH_3), 4.31 (t, $J=7.1$, 2H, CH_2), 8.32 (s, 1H, 2-H), 8.38 (s, 1H, 8-H). Anal. Found: $C_{18}H_{31}N_5\cdot HCl$ (C, H, N).

*5.1.46. 9-Phenethyl-N^6-methyladenine hydrochloride (**9f**)*

The title compound was prepared from **8a** and phenethyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, DMSO-d_6) δ 2.95 (br s, 3H, CH_3), 3.14 (t, $J=7.3$, 2H, CH_2), 4.39 (t, $J=7,3$, 2H, CH_2), 7.12–7.26 (m, 5H, 5 CH), 7.61 (br s, 1H, NH), 7.91 (s, 1H, 2-H), 8.23 (s, 1H, 8-H). Anal. Found: $C_{14}H_{15}N_5\cdot HCl\cdot 0.2H_2O$ (C, H, N).

*5.1.47. N^6-methyl-9-(3-phenylpropyl)adenine hydrochloride (**9g**)*

The title compound was prepared from **8a** and 3-phenylpropyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, CD_3OD) δ 2.19–2.33 (m, 2H, CH_2), 2.68 (t, $J = 7.7$, 2H, CH_2), 3.21 (s, 3H, CH_3), 4.35 (t, $J = 7.1$, 2H, CH_2), 7.06–7.27 (m, 5H, 5CH), 8.34 (s, 1H, 2-H), 8.39 (s, 1H, 8-H). Anal. Found: $C_{15}H_{17}N_5 \cdot HCl \cdot 1.2H_2O$ (C, H, N).

*5.1.48. 9-(4-Chlorobenzyl)-N^6-methyladenine hydrochloride (**9h**)*

The title compound was prepared from **8a** and 4-chlorobenzyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, DMSO-d_6) δ 2.94 (d, $J = 4.8$, 3H, CH_3), 5.41 (s, 2H, CH_2), 7.21–7.29 (m, 4H, 4CH), 8.35 (s, 1H, 2-H), 8.89 (s, 1H, 8-H), 9.78 (q, $J = 4.8$, 1H, NH). Anal. Found: $C_{13}H_{12}ClN_5 \cdot HCl \cdot 0.4H_2O$ (C, H, N).

*5.1.49. 9-(2-Bromobenzyl)-N^6-methyladenine hydrochloride (**9i**)*

The title compound was prepared from **8a** and 2-bromobenzyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.21 (d, $J = 4.8$, 3H, CH_3), 5.47 (s, 2H, CH_2), 5.97 (br s, 1H, NH), 7.09–7.29 (m, 3H, 3CH), 7.58–7.63 (m, 1H, CH), 7.80 (s, 1H, 8-H), 8.44 (s, 1H, 2-H). Anal. Found: $C_{13}H_{12}BrN_5 \cdot HCl$ (C, H, N).

*5.1.50. 9-(2-Methoxybenzyl)-N^6-methyladenine hydrochloride (**9j**)*

The title compound was prepared from **8a** and 2-methoxybenzyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.03 (br s, 3H, CH_3), 3.77 (s, 3H, CH_3), 5.35 (s, 2H, CH_2), 6.81–7.31 (m, 4H, 4CH), 8.39 (s, 2H, 2-H + 8-H), 9.59 (br s, 1H, NH). Anal. Found: $C_{14}H_{15}N_5O \cdot HCl \cdot 0.2H_2O$ (C, H, N).

*5.1.51. 9-(4-Methoxybenzyl)-N^6-methyladenine hydrochloride (**9k**)*

The title compound was prepared from **8a** and 4-methoxybenzyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.06 (d, $J = 4.0$, 3H, CH_3), 3.66 (s, 3H, CH_3), 5.36 (s, 2H, CH_2), 6.88–7.28 (m, 4H, 4 CH), 8.43 (s, 1H, 2-H), 8.60 (s, 1H, 8-H), 9.85 (br s, 1H, NH). Anal. Found: $C_{14}H_{15}N_5O \cdot HCl \cdot 0.4H_2O$ (C, H, N).

*5.1.52. N^6-methyl-9-(2-oxo-2-phenethyl)adenine hydrochloride (**9l**)*

The title compound was prepared from **8a** and α-chloro acetophenone following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.11 (s, 3H, CH_3), 6.03 (s, 2H, CH_2), 7.58–7.79 (m, 3H, 3 CH), 8.07–8.12 (m, 2H, 2CH), 8.39 (s, 1H, 2-H), 8.41 (s, 1H, 8-H), 9.60 (br s, 1H, NH). Anal. Found: $C_{14}H_{13}N_5O \cdot HCl \cdot 0.5H_2O$ (C, H, N).

*5.1.53. N^6-methyl-9-(3,3-diphenylpropyl)adenine hydrochloride (**9m**)*

The title compound was prepared from **8a** and 3,3-diphenylpropyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, CD_3OD) δ 2.68–2.75 (m, 2H, CH_2), 3.18 (s, 3H, CH_3), 3.92–4.03 (m, 1H, CH), 4.31 (t, $J = 7.1$, 2H, CH_2), 7.10–7.25 (m, 10H, 10CH), 8.17 (s, 1H, 2-H), 8.35 (s, 1H, 8-H). Anal. Found: $C_{21}H_{21}N_5 \cdot HCl$ (C, H, N).

*5.1.54. 9-[(4-Benzoyl)benzyl]-N^6-methyladenine hydrochloride (**9n**)*

The title compound was prepared from **8a** and 4-benzoylbenzyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, DMSO-d_6) δ 3.10 (br s, 3H, CH_3), 5.61 (s, 2H, CH_2), 7.43–7.71 (m, 9H, 9CH), 8.46 (s, 1H, 2-H), 8.69 (s, 1H, 8-H), 9.92 (br s, 1H, NH). Anal. Found: $C_{20}H_{17}N_5O \cdot HCl$ (C, H, N).

*5.1.55. 9-Benzyl-N^6-methyl-2-n-pentyladenine hydrochloride (**9o**)*

The title compound was prepared from **8b** and benzyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, CD_3OD) δ 0.93 (t, $J = 6.8$, 3H, CH_3), 1.32–1.48 (m, 4H, $2CH_2$), 1.83–1.91 (m, 2H, CH_2), 2.84–3.18 (t, $J = 7.7$, 2H, CH_2), 3.22 (s, 3H, CH_3), 5.47 (s, 2H, CH_2), 7.29–7.39 (m, 5H, 5CH), 8.30 (s, 1H, 8-H). Anal. Found: $C_{18}H_{23}N_5 \cdot HCl$ (C, H, N).

*5.1.56. 9-Benzyl-N^6-methyl-2-phenethyladenine hydrochloride (**9p**)*

The title compound was prepared from **8d** and benzyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, CD_3OD) δ 2.89 (s, 3H, CH_3), 4.90–5.01 (m, 4H, $2CH_2$), 5.47 (s, 2H, CH_2), 7.15–7.28 (m, 5H, 5CH), 7.29–7.40 (m, 5H, 5CH), 8.32 (s, 1H, 8-H). Anal. Found: $C_{21}H_{21}N_5 \cdot HCl \cdot 2H_2O$ (C, H, N).

*5.1.57. 9-Benzyl-N^6-methyl-2-(3-phenylpropyl)adenine hydrochloride (**9q**)*

The title compound was prepared from **8e** and benzyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.19–2.34 (m, 2H, CH_2), 2.75 (t, J = 7.5, 2H, CH_2), 3.02 (t, J = 7.5, 2H, CH_2), 3.59 (d, J = 5.1, 3H, CH_3), 5.34 (s, 2H, CH_2), 7.11–7.42 (m, 10H, 10CH), 7.88 (s, 1H, 8-H), 9.35 (br s, 1 H, NH). Anal. Found: $C_{22}H_{23}N_5 \cdot HCl \cdot 1.3H_2O$ (C, H, N).

*5.1.58. 2-Trifluoromethyl-9-(2-methoxybenzyl)-N^6-methyladenine (**9r**)*

The title compound was prepared from **8f** and 2-methoxybenzyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.19 (br s, 3H, CH_3), 3.83 (s, 3H, CH_3), 5.34 (s, 2H, CH_2), 5.85 (br s, 1H, NH), 6.85–6.94 (m, 2H, 2CH), 7.23–7.33 (m, 2H, 2CH), 7.84 (s, 1H, 8-H). Anal. Found: $C_{15}H_{14}F_3N_5O \cdot 0.2H_2O$ (C, H, N).

*5.1.59. 9-(2-Methoxybenzyl)-N^6-methyl-2-n-propyladenine hydrochloride (**9s**)*

The title compound was prepared from **8g** and 2-methoxybenzyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.03 (t, J = 7.3, 3H, CH_3), 1.85–2.06 (m, 2H, CH_2), 2.92 (t, J = 7.5, 2H, CH_2), 3.47 (d, J = 4.7, 3H, CH_3), 3.88 (s, 3H, CH_3), 5.34 (s, 2H, CH_2), 6.90–6.99 (m, 2H, 2CH), 7.27–7.39 (m, 2H, 2CH), 7.97 (s, 1H, 8-H), 9.28 (br s, 1H, NH). Anal. Found: $C_{17}H_{21}N_5O \cdot HCl \cdot 0.5H_2O$ (C, H, N).

*5.1.60. 9-[(4-Benzoyl)benzyl]-N^6-methyl-2-n-propyladenine hydrochloride (**9t**)*

The title compound was prepared from **8g** and 4-benzoylbenzyl chloride following the general procedure for the alkylation of N^6-methyladenines (method B): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.03 (t, J = 7.3, 3H, CH_3), 1.86–2.01 (m, 2H, CH_2), 2.95 (t, J = 7.5, 2H, CH_2), 3.62 (d, J = 5.1, 3H, CH_3), 5.48 (s, 2H, CH_2), 7.40–7.84 (m, 9H, 9CH), 7.98 (s, 1H, 8-H), 9.45 (br s, 1H, NH). Anal. Found: $C_{23}H_{23}N_5O \cdot HCl \cdot 0.5H_2O$ (C, H, N).

*5.1.61. 9-Benzyl-2-iodo-N^6-methyladenine hydrochloride (**13a**)*

To a solution of 2-amino-9-chloropurine **10** (1.0 g, 5.90 mmol), K_2CO_3 (815 mg, 5.90 mmol), and tetra-*n*-butylammonium iodide (100 mg, 0.27 mmol) in dry DMF (8.0 mL) was added dropwise benzyl chloride (815 μl, 7.08 mmol). The resulting suspension was stirred for 12 h at room temperature. After evaporation of the solvent, the residue was diluted with cold water (20 mL) and extracted three times with ethyl acetate (30 mL). Organic layers were dried (Na_2SO_4) and concentrated to dryness under reduced pressure. Chromatography on silica (AcOEt) afforded compound **11a** (930 mg, 60%) as a colorless solid: ^{1}H-NMR (200 MHz, $CDCl_3$) δ 5.00 (br s, 2H, NH_2), 5.31 (s, 2H, CH_2), 7.30–7.32 (m, 5H, 5CH), 8.09 (s, 1H, 8-H).

A solution of **11a** (270 mg, 1 mmol), isoamyle nitrite (2.69 mL, 20 mmol) and diiodomethane (10 mL, 124 mmol) was stirring at 85 °C under argon for 1 h. After cooling, volatiles were evaporated, and the residue was taken up in AcOEt. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by chromatography on silica. Elution with CH_2Cl_2–AcOEt (90:10) delivered **12a** as an amorphous solid (260 mg, 70%) which was used in the next step without further purification: ^{1}H-NMR (200 MHz, $CDCl_3$) δ 5.40 (s, 2H, CH_2), 7.26–7.41 (m, 5H, 5CH), 7.98 (s, 1H, 8-H).

A solution of **12a** (200 mg, 0.54 mmol) and methylamine (1.0 M in THF, 4 mL) in absolute ethanol (4 mL) was stirred for 24 h at room temperature. After evaporation of the solvent, the residue was chromatographed on silica (AcOEt–CH_2Cl_2–EtOH, 4:5:1), then recrystallized from ethanol to give **13a** (137 mg, 63%) as a white solid: ^{1}H-NMR (200 MHz, CD_3OD) δ 3.15 (s, 3H, CH_3), 5.51 (s, 2H, CH_2), 7.36–7.44 (m, 5H, 5CH), 8.96 (s, 1H, 8-H). Anal. Found: $C_{13}H_{12}IN_5 \cdot HCl$ (C, H, N).

*5.1.62. 2-Iodo-9-(2-methoxybenzyl)-N^6-methyladenine hydrochloride (**13b**)*

Prepared from **10** and 2-methoxybenzyl chloride using the procedure described for compound **13a**: ^{1}H-NMR (200 MHz, $CDCl_3$) δ 3.86 (s, 3H, CH_3), 5.36 (s, 2H, CH_2), 6.88–7.00 (m, 2H, 2CH), 7.26–7.38 (m, 2H, 2CH), 8.05 (s, 1H, 8-H). Anal. Found: $C_{14}H_{14}IN_5O \cdot HCl$ (C, H, N).

*5.1.63. 9-Benzyl-N^6-methyl-2-prop-1-ynyladenine hydrochloride (**14a**)*

Methyl acetylene (2 mL) was condensed in a sealed tube at −78 °C. Then **13a** (200 mg, 0.55 mmol), CuI (10 mg, 0.055 mmol), $PdCl_2$ (6 mg, 0.034 mmol), triphenylphosphine (20 mg, 0.076 mmol), triethylamine (2 mL, 14.3 mmol) and acetonitrile (4 mL) were added. The solution was stirred at room temperature for 24 h, the solvent was then removed under vacuum, and the residue was purified by flash chromatography (CH_2Cl_2–AcOEt, 80:20) and converted to an HCl salt. Recrystallization from ethanol and diethyl ether yielded compound **14a** (121 mg, 70%): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.20 (s, 3H, CH_3), 3.61 (d, J = 5.5, 3H, CH_3), 5.37 (s, 2H, CH_2), 7.27–7.44 (m, 5H, 5CH), 7.88 (s, 1H, 8-H), 9.82 (br s, 1H, NH). Anal. Found: $C_{16}H_{15}N_5 \cdot HCl$ (C, H, N).

*5.1.64. 9-(2-Methoxybenzyl)-N^6-methyl-2-prop-1-ynyladenine hydrochloride (**14b**)*

Prepared from **13b** using the procedure described for compound **14a**: ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.20 (s, 3H, CH_3), 3.59 (d, $J = 5.5$, 3H, CH_3), 3.88 (s, 3H, CH_3), 5.35 (s, 2H, CH_2), 6.92–7.04 (m, 2H, 2CH), 7.34–7.55 (m, 2H, 2CH), 7.99 (s, 1H, 8-H), 9.77 (br s, 1H, NH). Anal. Found: $C_{17}H_{17}N_5O \cdot HCl \cdot 1.6H_2O$ (C, H, N).

*5.1.65. trans-9-(2-Methoxybenzyl)-N^6-methyl-2-prop-1-enyladenine hydrochloride (**14c**)*

To a solution of **13b** (150 mg, 0.35 mmol), *tetrakis*-triphenylphosphine palladium (4 mg, 3.46 10^{-3} mmol) in CH_3CN (10 mL) was added 6 N sodium hydroxide (400 µl) and (*E*)-prop-1-enylcatecholborane [38] (320 mg, 2 mmol). The reaction mixture was refluxed under argon for 4 h. After cooling, the solvent was removed under vacuum and the residue was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (AcOEt) and converted to an HCl salt. Recrystallization from ethanol and diethyl ether gave **14c** (87 mg, 72%) as colorless prisms: ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.05 (dd, $J = 6.9$, $J = 1.7$, 3H, CH_3), 3.59 (d, $J = 5.1$, 3H, CH_3), 3.89 (s, 3H, CH_3), 5.34 (s, 2H, CH_2), 6.45–6.54 (m, 1H, CH), 6.92–7.01 (m, 2H, 2CH), 7.33–7.41 (m, 1H, CH), 7.46–7.65 (m, 2H, 2CH), 7.96 (s, 1H, 8-H), 9.55 (br s, 1H, NH). Anal. Found: $C_{17}H_{19}N_5O \cdot HCl$ (C, H, N).

*5.1.66. 9-(2-Methoxybenzyl)-N^6-methyl-2-methylmercaptoadenine hydrochloride (**14d**)*

A solution of **13b** (43 mg, 0.10 mmol), sodium methanethiolate (350 mg, 0.50 mmol) in dimethylformamide (3 mL) and ethanol (3 mL) was stirring at 120 °C under argon in a sealed tube for 6 h. After cooling, the solvent was removed under vacuum, and the residue was purified by flash chromatography (AcOEt–EtOH, 90:10). Transformation into the HCl salt and recrystallization from EtOH and diethyl ether gave **14d** (31 mg, 88%) as colorless prisms: ^{1}H-NMR (200 MHz, $CDCl_3$) δ 2.62 (s, 3H, CH_3), 3.14 (d, $J = 4.8$, 3H, CH_3), 3.90 (s, 3H, CH_3), 5.41 (s, 2H, CH_2), 6.93–7.04 (m, 2H, 2CH), 7.33–7.51 (m, 2H, 2CH), 8.38 (s, 1H, 8-H), 8.56 (br s, 1H, NH). Anal. Found: $C_{15}H_{17}N_5OS \cdot HCl$ (C, H, N).

5.2. Pharmacology

5.2.1. PDE inhibition

PDE-4 was isolated from the media layer of bovine aorta according to a modification of the previously reported method [34]. PDE activities were measured by the two-step assay previously described [35] at a [^{3}H]-cAMP or [^{3}H]-cGMP concentration of 1 µM as substrate in a buffer solution of 50 mM of Tris–HCl, at pH 7.5, containing 2 mM of magnesium acetate, 1 mg mL^{-1} of BSA. PDE-1 was assayed at 1 µM cGMP in calmodulin activated state (18 nM calmodulin with 10 µM $CaCl_2$). PDE-2 was evaluated at 1 µM cAMP + 1 mM EGTA in activated state (in presence of 5 µM cGMP). PDE-3 and PDE-4 were assayed at 1 µM cAMP + 1 mM EGTA. To prevent the influence of reciprocal cross-contamination between PDE-3 and PDE-4, the studies were always carried out in the presence of 50 µM rolipram for PDE-3 and in the presence of 50 µM cGMP for PDE-4. PDE-5 activity was measured at 1 µM cGMP in the presence of 1 mM of EGTA. PDE inhibitors were dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 1%. At this concentration, DMSO had no significant effects on PDE activity. The concentration of drugs that produced 50% inhibition of substrate hydrolysis (IC_{50}) was calculated by nonlinear regression analysis from concentration–response curves and included different concentrations of inhibitors. The results represent the mean of three determinations obtained for three different enzymatic preparations. The experimental error is about 15%.

5.2.2. Subjects

Healthy donors who had no clinical history were selected for this study. This work was approved by the local ethics committee (CCPPRB d'Alsace no. 1), and carried out according national guidelines.

5.2.3. Peripheral blood mononuclear cells

Healthy subjects PBMCs were separated as previously described [36]. Briefly, peripheral blood diluted with Hank's balanced salt solution, Ca^{2+} and Mg^{2+} free, containing 100 IU heparin mL^{-1} was layered over Histopaque-1077 (Sigma, St. Louis, MO), and centrifuged for 30 min at $400 \times g$ (20 °C). Cells harvested from the interface were washed 3 times in HBSS-CMF and resuspended at a final concentration of 2×10^6 ml^{-1} in a culture medium. Human PBMCs were incubated with increasing doses of the tested drugs ranging from 10^{-8} to 10^{-5} M, with or without activation by lipopolysacharide from *Salmonella abortus equi* (Sigma, L'Isle d'Abeau Chesnes, France) in 24 well culture plates (Falcon, Poly Labo, Strasbourg, France) for 24 h at 37 °C in a humidified 5% CO_2–95% air atmosphere. After incubation, supernatants were removed and stored at −80 °C until ELISA. Cell viability was assessed by the Trypan blue exclusion test.

5.2.4. Immunoassays for TNFα

Culture supernatants were assayed with two-site ELISAs specific for human interleukin: TNFα antibo-

dies (Antibody Solutions, Half Moon Bay, CA). Quantitative evaluation of PBMCs secreted TNFα was achieved by ELISA using conditions as previously described [37]. Polyvinyl chloride plates (Costar, # 2596) were coated with 50 µl per well of antibodies (15 µg mL^{-1}) and incubated overnight at 4 °C. After the usual wash and non-specific saturation steps, 25 µl of standard or sample were added to 25 µl of biotinylated monoclonal antibody (2 µg mL^{-1}) for 2 h at room temperature. Following washing steps, 50 µl of peroxidase streptavidin dilution (1:3000 in PBS) were added (1 h at room temperature). A colorimetric reaction (O.D. at 450 nm) using *O*-phenyl-enediamine as peroxidase substrate, was performed ensuing four washing steps. Concentrations (pg mL^{-1}) of unknown samples were computed by interpolation with a standard curve run on each plate using four parameters logistics analysis. Standard human recombinant protein, hr-TNFα, was purchased from R&D Systems Europe (Abingdon, UK).

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Appendix A. Supporting information

Product	Formula	C (%)		H (%)		N (%)	
		Calculated	Found	Calculated	Found	Calculated	Found
7a	$C_{12}H_{12}N_6 \cdot 2HCl$	46.02	46.18	4.50	4.51	26.83	26.69
7b	$C_{12}H_{11}N_5O \cdot HCl \cdot 1H_2O$	48.74	48.89	4.77	4.49	23.68	23.64
7c	$C_{16}H_{17}N_5 \cdot HCl \cdot 1H_2O$	57.57	57.39	6.04	5.87	20.98	21.13
7d	$C_{16}H_{18}N_6 \cdot 2HCl$	52.32	52.42	5.48	5.45	22.88	22.67
7e	$C_{13}H_{13}N_50$	61.17	61.34	5.13	5.04	27.43	27.31
7f	$C_{13}H_{12}ClN_5 \cdot HCl \cdot 0.7H_2O$	48.37	48.42	4.50	4.40	21.69	21.43
7g	$C_{14}H_{13}N_5O_2 \cdot HCl$	52.59	52.61	4.41	4.38	21.90	21.81
7h	$C_{15}H_{15}N_5O_2 \cdot HCl \cdot 1.6H_2O$	49.68	49.68	4.89	4.78	19.31	19.42

Appendix (*Continued*)

Product	Formula	C (%)		H (%)		N (%)	
		Calculated	Found	Calculated	Found	Calculated	Found
7i	$C_{15}H_{16}N_6O \cdot HCl \cdot 2.1H_2O$	48.61	48.61	5.19	5.14	22.67	22.43
7j	$C_{14}H_{15}N_5O \cdot HCl$	51.41	51.14	5.27	5.40	22.90	22.62
7k	$C_{12}H_{11}N_5 \cdot HCl \cdot 0.2H_2O$	54.70	54.63	4.30	4.62	26.41	26.11
7l	$C_{16}H_{19}N_5 \cdot HCl$	60.46	60.41	6.34	6.29	22.03	22.37
7m	$C_{16}H_{19}N_5 \cdot HCl$	60.46	60.32	6.32	6.35	22.03	22.17
7n	$C_{19}H_{23}N_5 \cdot HCl$	63.77	63.98	6.76	6.74	19.57	19.75
7o	$C_{17}H_{21}N_5 \cdot HCl$	61.52	61.24	6.68	6.63	21.10	20.99
7p	$C_{21}H_{19}N_5 \cdot HCl$	66.75	66.53	5.33	5.12	18.53	18.78
7q	$C_{13}H_{14}N_6 \cdot 2HCl$	47.70	47.98	4.93	5.03	25.68	25.70
7r	$C_{14}H_{13}F_3N_6O$	49.71	49.62	3.87	3.67	24.84	25.98
7s	$C_{15}H_{17}N_5O \cdot HCl$	56.33	56.31	5.67	5.81	21.90	21.79
7t	$C_{15}H_{17}N_5O \cdot HCl$	56.33	56.25	5.67	5.50	21.90	21.69
7u	$C_{16}H_{19}N_5O \cdot HCl$	57.57	57.72	6.04	6.28	20.98	21.12
7v	$C_{12}H_{19}N_5O_2 \cdot HCl \cdot 1.5H_2O$	43.84	43.71	7.05	7.30	21.30	21.42
9a	$C_{13}H_{13}N_5 \cdot HCl \cdot 0.5H_2O$	54.83	54.98	5.31	5.27	24.59	24.81
9b	$C_{10}H_{15}N_5 \cdot HCl \cdot 0.3H_2O$	48.60	48.56	6.36	6.57	28.34	28.33
9c	$C_{11}H_{15}N_5 \cdot HCl \cdot 2.3H_2O$	44.76	44.80	7.03	7.03	23.73	23.69
9d	$C_{11}H_{17}N_5 \cdot HCl \cdot 1.5H_2O$	46.73	46.46	7.48	7.52	24.76	24.60
9e	$C_{18}H_{31}N_5 \cdot HCl$	61.08	60.74	9.11	9.13	19.79	19.88
9f	$C_{14}H_{15}N_5 \cdot HCl \cdot 0.2H_2O$	57.32	57.14	5.63	5.57	23.87	23.47
9g	$C_{15}H_{17}N_5 \cdot HCl \cdot 1.2H_2O$	55.36	55.24	6.32	6.32	21.52	21.60
9h	$C_{13}H_{12}ClN_5 \cdot HCl \cdot 0.4H_2O$	49.20	49.49	4.38	4.32	22.07	21.97
9i	$C_{13}H_{12}BrN_5 \cdot HCl$	44.03	43.80	3.69	3.77	19.75	19.78
9j	$C_{14}H_{15}N_5O \cdot HCl \cdot 0.2H_2O$	54.35	54.20	5.34	5.38	22.64	22.63
9k	$C_{14}H_{15}N_5O \cdot HCl \cdot 0.4H_2O$	53.73	53.89	5.41	5.30	22.38	22.51
9l	$C_{14}H_{13}N_5O \cdot HCl \cdot 0.5H_2O$	53.76	53.72	4.83	4.59	22.39	22.60
9m	$C_{21}H_{21}N_5 \cdot HCl$	66.40	66.70	5.84	5.94	18.44	18.45
9n	$C_{20}H_{17}N_5O \cdot HCl$	63.24	63.00	4.77	4.81	18.43	18.28
9o	$C_{18}H_{23}N_5 \cdot HCl$	62.50	62.11	6.70	6.99	20.24	20.16
9p	$C_{21}H_{21}N_5 \cdot HCl \cdot 2H_2O$	60.64	60.54	6.30	6.48	16.83	16.84
9q	$C_{22}H_{23}N_5 \cdot HCl \cdot 1.3H_2O$	63.31	63.35	6.18	6.01	16.78	16.99
9r	$C_{15}H_{14}F_3N_5O \cdot 0.2H_2O$	52.84	52.93	4.26	4.24	20.54	20.33
9s	$C_{17}H_{21}N_5O \cdot HCl \cdot 0.5H_2O$	57.22	57.20	6.44	6.59	19.62	19.73
9t	$C_{23}H_{23}N_5O \cdot HCl \cdot 0.5H_2O$	64.11	64.08	5.85	5.72	16.25	16.38
13a	$C_{13}H_{12}IN_5 \cdot HCl$	38.88	39.10	3.26	3.35	17.43	17.08
13b	$C_{14}H_{14}IN_5O \cdot HCl$	38.95	39.15	3.50	3.62	16.22	16.15
14a	$C_{16}H_{15}N_5 \cdot HCl$	55.80	55.73	5.67	5.66	20.33	20.24
14b	$C_{17}H_{17}N_5O \cdot HCl \cdot 1.6H_2O$	51.32	51.13	5.57	5.52	17.60	17.37
14c	$C_{17}H_{19}N_5O \cdot HCl$	59.04	58.87	5.83	5.78	20.25	20.13
14d	$C_{15}H_{17}N_5OS \cdot HCl$	51.20	51.16	5.16	5.03	19.90	19.98