9-Benzyladenines: Potent and Selective cAMP Phosphodiesterase Inhibitors

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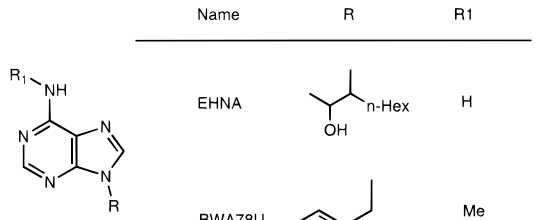
Adenine derivatives substituted in position 9 constitute an important class of pharmacologically active compounds for which different targets can be postulated. Because they resemble the structure of adenosine, they may compete with the neuromodulator at its specific pharmacological receptors,[1] transport proteins, or metabolic enzymes. Particularly *erythro*-9-(2-hydroxy-3-nonyl)adenine (EHNA) is known as a potent inhibitor of adenosine deaminase[2] (Figure 1).

However, 9-substituted adenines also constitute possible competitors for adenosine-deriving endogenous substances, such as adenosine monophosphate and its corresponding cyclic nucleotide, diphosphate, and triphosphate (AMP, cAMP, ADP, ATP) respectively. Thus EHNA was found to inhibit a specific phosphodiesterase (PDE) isozyme found in heart[3] and vascular endothelial cells[4] with micromolar IC_{50} values.

Several years ago, Kelley *et al*. described a 9-benzyladenine derivative (BWA78U) possessing potent anticonvulsant effects.[5] As this compound also presents potent anxiolytic and sedative properties,[6] an interaction with benzodiazepine receptors has been first suspected, but found to be very weak[7,8] ([^{3}H]diazepam, IC_{50} = 13 μM). Moreover, a pretreatment of the rats with the benzodiazepine receptor antagonist flumazenil did not block the potent anticonvulsant properties of this compound (Marescaux and Bourguignon, unpublished results). As BWA78U did not bind to A1 and A2 adenosine receptors ([^{3}H]CHA and [^{3}H]NECA, IC_{50} > 100 μM), we evaluated its capacity to inhibit the different phosphodiesterases (PDE), particularly those hydrolyzing cAMP. Following the classification proposed by Beavo,[9] the different PDE isoforms can be grouped into seven families according to the related gene, their substrate specificity, their modulation by endogenous regulators (calcium−calmodulin, cGMP), and their selective inhibitions by typical inhibitors. In this paper, we report the behavior of BWA78U and some of its analogues toward PDE3 and PDE4 preparations. It appears that some of these compounds exhibit potent phosphodiesterase inhibition properties with high selectivity toward PDE4.

Chemistry. The 2-substituted 6-chloropurines **2a**−**c** were obtained from their corresponding hypoxanthines[10−12] by treatment with $POCl_3$ in presence of *N*,*N*-dimethylaniline (Scheme 1).[13] Deprotonation of 6-chloropurines **2** by means of potassium carbonate in DMF or DMSO, and subsequent alkylation with different akyl halides, afforded the corresponding 9-substituted isomers **3** as major compounds. The 6-chloro-4,5-diaminopyrimidines **4**, obtained as described earlier,[5] were reacted with methyl orthoacetate in presence of catalytic amounts of methanesulfonic acid to yield the corresponding 8-methylchloropurines **5**. Treatment of the latter compounds **3** or **5** with various amines provided the desired adenines **6** (NCS compounds). The most basic adenines were analyzed and tested as their hydrochlorides or their more water-soluble methanesulfonate salts.



Ki (μM)

Name	PDE1	PDE2	PDE3	PDE4	PDE5
EHNA	ns	2.8	ns	ns	ns
BWA78U	3.7	122	35	2.0	6.5
Rolipram	ns	ns	ns	0.8	ns

ns : Ki > 200 μM

Figure 1. Phosphodiesterase inhibition of two known pharmacologically active 9-substituted adenines.

The efficiencies of a first series of differently substituted 9-alkyladenines have been evaluated as PDE inhibitors via their IC_{50} values on PDE3 and PDE4 from vascular smooth muscle. The concentration of each drug that inhibited 50% of the enzymatic activity was determined at 1 μM substrate concentration. Data are listed in Table 1.

Results and Discussion

PDE1−PDE5 are well-known and pharmacologically characterized. PDE1 is activated by calcium−calmodulin and preferentially hydrolyzes cGMP in brain and in vascular smooth muscle. The PDE2 which hydrolyzes both cAMP and cGMP is activated by cGMP. Activated PDE2 is specifically inhibited by EHNA. This isoform is present in brain and vascular endothelial cells. PDE3 which preferentially hydrolyzes cAMP is inhibited by cGMP and specific cardiotonic agents (SKF 94120). PDE3 is present in platelets, cardiac tissue, and vascular smooth muscle but is absent in brain. PDE4 specifically hydrolyzes cAMP, is potently and specifically inhibited by rolipram, and is present in brain, cardiac tissue, and vascular smooth muscle. PDE5 specifically hydrolyzes cGMP and is preferentially inhibited by zaprinast. PDE5 is present in vascular and airway smooth muscle tissues but is absent in the brain and heart.

We found that BWA78U was able to significantly inhibit several PDE isoforms from vascular smooth muscle, particularly its K_i value of 2 μM for PDE4 could be compared with that of rolipram (K_i = 0.8 μM), the reference inhibitor for this isoform (Figure 1). Unexpectedly, when considering K_i values, it presented some

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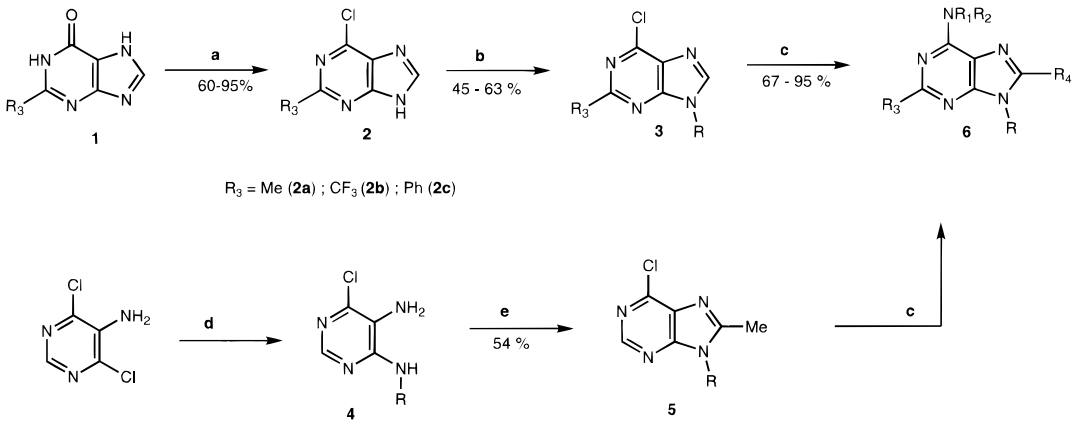
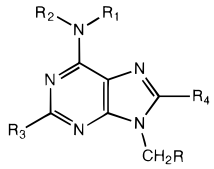
Scheme 1. Access to Differently Substituted Adenine Derivatives[a]



[a] (a) $POCl_3$; (b) RCl (or RBr), K_2CO_3, DMSO; (c) HNR_1R_2, EtOH; (d) RNH_2, BuOH, Et_3N, reflux; (e) Me-C(OMe)$_3$, H^+, room temperature.

Table 1. Inhibition of PDE's by 9-Alkyladenines



compd	salt	mp, °C	R_1	R_2	R_3	R_4	R	IC_{50}[a] (μM) PDE$_3$	IC_{50}[a] (μM) PDE$_4$
BWA78U[a]	$MeSO_3H$	195	H	Me	H	H	2-FC_6H_4	121	3
6a	HCl	186	H	*n*-Bu	H	H	2-FC_6H_4	ns	53
6b	HCl	177	$-(CH_2)_5-$		H	H	2-FC_6H_4	ns	121
6c	$MeSO_3H$	146	H	Me	CH_3	H	2-FC_6H_4	250	0.2
6d	free base	138	H	Me	CF_3	H	2-FC_6H_4	380	0.04
6e	$MeSO_3H$	188 dec	H	Me	Ph	H	2-FC_6H_4	ns	0.5
6f	$MeSO_3H$	176	H	Me	H	H	C_6H_5	339	4.6
6g	free base	233	H	H	H	H	C_6H_5	ns	160
6h	$MeSO_3H$	156	H	Me	H	Me	2-FC_6H_4	ns	54
6i	free base	175	H	H	CF_3	H	C_6H_5	101	1.8
6j	free base	109	Me	Me	CF_3	H	C_6H_5	61	11
6k	free base	155	H	Me	CF_3	H	$CH_2C_6H_5$	ns	0.75
6l	free base	195	H	Me	CF_3	H	H	331	11

[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%. ns: $IC_{50} > 200$ μM.

selectivity profile, as it is significantly less active on PDE2 and PDE3 (K_i = 122 and 35 μM, respectively). Thus we focused our attention on both PDE4 and PDE3 and checked substituent effects on different positions of the adenine ring of BWA78U.

It is noteworthy that the fluorine in position 2 of the phenyl ring is not needed for PDE4 inhibition (compare BWA78U and compound **6f**). Methyl substituent effects allowed a first evaluation of minimal structural perturbations tolerated. Thus the presence of a methyl group in position 8 (**6h**) led to a more than 1 order of magnitude decrease in potency of this compound, when compared to BWA78U.

Introducing a methyl group in position 2 in compound **6c** significantly increased its potency. Thus the 2-methyl group was replaced by a trifluoromethyl (**6d**) or a phenyl group (**6e**). Strong beneficial effects were observed with the trifluoromethyl derivative **6d** (IC_{50} = 40 nM). This compound served as reference for further SAR studies. The *N*,*N*-dimethyl derivative **6j** was found to be 300 times less potent and less selective than **6d**, whereas the N,N-unsubstituted derivative **6i** was equipotent with BWA78U (micromolar range IC_{50} value). Suppressing the phenyl ring at position 9 led to a dramatic loss in activity (compare **6l** and **6d**). The unsubstituted *N*-benzyladenine **6g** with an IC_{50} of 160 μM clearly highlights the necessity for potency of the presence of a methyl group on exocyclic nitrogen of the adenine.

Cyclic AMP analogues have been recently tested as PDE inhibitors.[14] Some of them, with limited structural modifications such as 2′-deoxy-cAMP, showed micromolar IC_{50} values on PDE3 and PDE4. However, it was unexpected to discover cAMP PDE inhibitors derived from ribose-free adenines, and with same micromolar IC_{50} values. First SAR analysis indicated a high potency of adenines in the presence of (i) a secondary amidine bearing a methyl group in position 6, (ii) a benzyl group in position 9, and (iii) a trifluoromethyl group in position 2. Compound **6d** (NCS 613) was found to be highly selective in inhibiting the PDE4 (IC_{50} values of 40, 380, 0.04, and 5 μM for PDE1, PDE3, PDE4, and PDE5, respectively) and thus constitutes an efficient tool for the study of PDE4 inhibition. When compared with the high-affinity rolipram binding sites, another site of action in PDE4 can be postulated for BWA78U and adenine derivatives. This site probably partially overlaps the cAMP binding site.[14] Further work including

detailed SAR analysis on different PDE isoforms and pharmacophore identification will help to understand better the mechanism of action of this novel class of PDE4 inhibitors, in relation with their *in vivo* pharmacological properties, particularly their CNS properties.[6,7,15,16]

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