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SYNTHESIS AND BIOLOGICAL ACTIVITY OF TRISUBSTITUTED ADENINES AS A_{2A} ADENOSINE RECEPTOR ANTAGONISTS

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□ *The discovery of new drugs for the treatment of neurodegenerative disorders, such as Parkinson's disease, has become an attractive field of research. Due to the regulation of D_2 receptor activity by A_{2A} adenosine receptor, potent and selective ligands of A_{2A} subtype could be useful tools to study neurodegenerative disorders. A series of 2,8-disubstituted-9-ethyladenine derivatives was synthesized and tested in binding affinity assay at human adenosine receptors. New compounds showed good affinity and selectivity at A_{2A} receptor versus the other subtypes. The introduction of a bromine atom in 8-position increased the affinity of these compounds, leading to ligands with K_i in the nanomolar range.*

Keywords Adenosine receptor ligands; adenosine receptor antagonits; A_{2A} antagonists; 9-ethylpurine derivatives; substituted adenines

INTRODUCTION

Adenosine (Ado) is an endogenous modulator of a variety of physiological and pathophysiological processes that acts through the interaction with specific membrane receptors termed A_1, A_{2A}, A_{2B}, and A_3.[1]

In particular, adenosine is deeply involved in the control of motor behaviour and substantial evidences indicate that adenosine A_{2A} receptor antagonists improve motor deficits in animal models of Parkinson's disease. For this reason development of potent and selective A_{2A} adenosine receptor antagonists has become an attractive field for the discovery of new drugs

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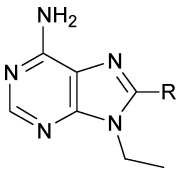


1 R = H
2 R = Br

FIGURE 1 Structure of 9-ethyl and 8-bromo-9-ethyladenines.

for the treatment of neurodegenerative disorders, such as Parkinson's disease.[2]

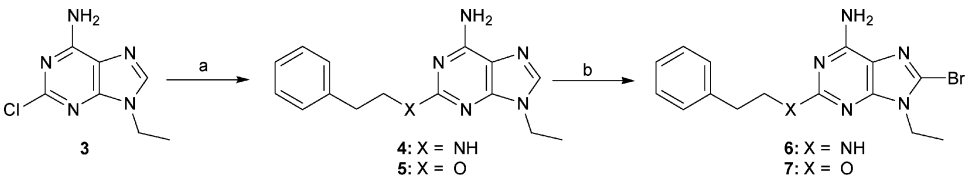
Ado receptor (AR) agonists are analogues of the natural ligand, whereas antagonists are characterized by a wide range of different structures. In particular, replacement of the ribose moiety of Ado with alkyl chains led to compounds that maintain affinity, but are not able to activate the receptors so behaving as antagonists.[3]

In a previous article we have reported the synthesis of a number of 9-ethylpurines bearing various substituents in 2-, 6-, or 8-position.[3] While 9-ethyladenine (**1**, Figure 1) showed micromolar affinity at the human A_1 and A_{2A} subtypes, the introduction of a bromine atom in 8-position led to an increase of binding affinity at all AR subtypes (see data for 9-ethyladenine (**1**) versus 8-bromo-9-ethyladenine (**2**) in Table 1). Furthermore, the substitution in the 2-position of **1** with a phenylethylamino (**4**) or a phenethoxy substituent (**5**) resulted in compounds endowed with increased A_{2A} affinity compared to **1** (Table 1).[3]

These observations prompted us to synthesize 9-ethyladenine derivatives substituted in 2-position with phenylalkylamino and phenylalkoxy groups and bearing a bromine atom in 8-position.

SYNTHESIS

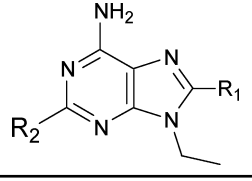
The synthesis of the 2-substituted 9-ethyladenines was carried out starting from the 2-chloro-9-ethyladenine (**3**; Figure 2), which was obtained from the 2,6-dichloropurine in two steps.[3]



a) $Ph(CH_2)_2NH_2$, ΔT; or $Ph(CH_2)_2OH$, NaOH, ΔT; b) NBS/DMF, rt.

FIGURE 2 Synthesis of 2,8-disubstituted-9-ethyladenines.

TABLE 1 Binding affinity of compounds **1–7** at the human A_1, A_{2A}, and A_3ARs subtypes and inhibition of NECA-stimulated adenylyl cyclase activity at the A_{2B} subtype



			K_i (nM)					
Cp	R_2	R_1	K_i (A_1)[a]	K_i (A_{2A})[a]	K_i (A_{2B})[a]	K_i (A_3)[a]	A_1/A_{2A}	A_3/A_{2A}
1	H	H	7,440 (4,220–13,120)	2,200 (1,400–3,530)	>30,000	>100,000	3	
2	H	Br	280 (250–320)	52 (24–110)	840 (630–1,100)	28,000 (22,000–35,000)	5	538
4	Ph-CH_2CH_2NH	H	330 (250–510)	150 (110–210)	2,400 (1,400–4,000)	3,200 (2,400–4,100)	2	21
6	Ph-CH_2CH_2NH	Br	150 (120–180)	19 (6–60)	690 (250–1,900)	3,100 (1,000–6,600)	8	163
5	Ph-CH_2CH_2O	H	170 (130–230)	120 (70–220)	45,800 (29,800–70,500)	7,150 (2,950–17,300)	1	60
7	Ph-CH_2CH_2O	Br	23 (23–24)	1.7 (1.4–2.2)	569 (440–734)	1,090 (685–1,720)	14	640

[a]95% confidence intervals in parentheses.

Treatment of the 2-chloro-9-ethyladenine (**3**) with the phenethylamine or phenethyl alcohol gave the corresponding 2-substituted derivatives **4**, **5**. The reactions were performed under high temperature and, in the second case, with addition of NaOH. 2-Substituted-9-ethyladenines were reacted with N-bromosuccinimide to obtain the 8-bromoderivatives **6** and **7**, (62% and 67% yield, respectively; Figure 2). Synthetic procedure and characterization of these compounds will be reported elsewhere.

BIOLOGICAL DATA

The new compounds were evaluated at the human recombinant ARs, stably transfected into Chinese hamster ovary (CHO) cells, utilizing radioligand binding studies (A_1, A_{2A}, A_3) or adenylyl cyclase activity assay (A_{2B}). Receptor binding affinity was determined using [^{3}H]CCPA as the radioligand for A_1 receptors, whereas [^{3}H]NECA was used for the A_{2A} and A_3 subtypes. In the case of A_{2B} receptors K_i-values were calculated from IC_{50} values determined by inhibition of NECA-stimulated adenylyl cyclase activity.[4]

Binding data showed that the new compounds **6** and **7** are endowed with good affinity for ARs and are slightly A_{2A} selective (Table 1). In fact, introduction of a bromine atom in 8-position improved affinity at all adenosine receptors, leading to compounds which showed affinity at A_{2A} receptor in the low nanomolar range and good selectivity for the A_{2A} versus A_3 subtype (**6**: $K_iA_{2A} = 19$ nM, $A_3/A_{2A} = 163$; **7**: $K_iA_{2A} = 1.7$ nM, $A_3/A_{2A} = 640$), the 2-phenethoxy derivative being the most active compound.

CONCLUSION

The newly synthesized trisubstituted adenines **6** and **7** are endowed with good affinity for the human A_{2A} adenosine receptor subtype; the 8-bromo-9-ethyl-2-phenethyloxy-9*H*-purine-6-ylamine (**7**), showing the highest A_{2A} affinity and selectivity, could be a starting point for searching new A_{2A} AR antagonists.

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