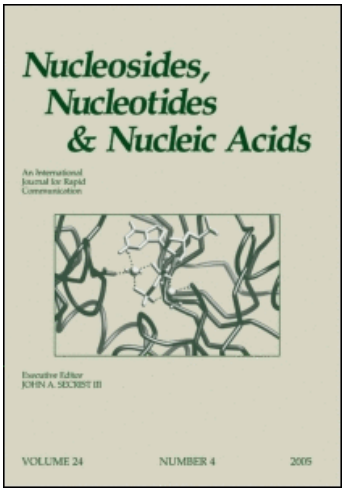

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POTENT AND SELECTIVE LIGANDS FOR ADENOSINE BINDING SITES

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Abstract. A number of selective ligands for the different binding sites of adenosine have been synthesized and tested in several pharmacological models. The aim of these synthetic efforts is both to improve the knowledge of structure-activity relationships in the adenosine-related biological systems and to develop drugs from some of these molecules.

Introduction

The purine nucleoside adenosine acts as a neurotransmitter or neuromodulator through the interaction with at least four cell surface receptors recently classified as A_1, A_{2A}, A_{2B}, and A_3.[1] These receptor subtypes have been cloned and characterized as belonging to the superfamily of receptors with seven transmembrane helices that couple to G proteins.[2]

In addition adenosine is substrate of many enzymes of the purine metabolism (adenosine deaminase, adenosine kinase, and 5'-nucleotidase), and the adenosine structure is also part of other bioactive molecules (ATP, ADP, cAMP, NAD, FAD, S-adenosyl methionine etc.).[3]

The clinical usefulness of adenosine has been recognised in the United States in the 1980s[4] and now adenosine is commonly used in the diagnosis and treatment of supraventricular arrhythmias.[5] However, the fact that adenosine is present throughout the body in all organs is a severe problems in the development of drugs acting specifically at the different adenosine binding sites.

Therefore our synthetic efforts during the last decade were devoted to the acquisition of structure-activity relationships and to the development of potent and selective agonists for adenosine binding sites. Some of successful ligands synthesized in our laboratory are listed in Figure 1.

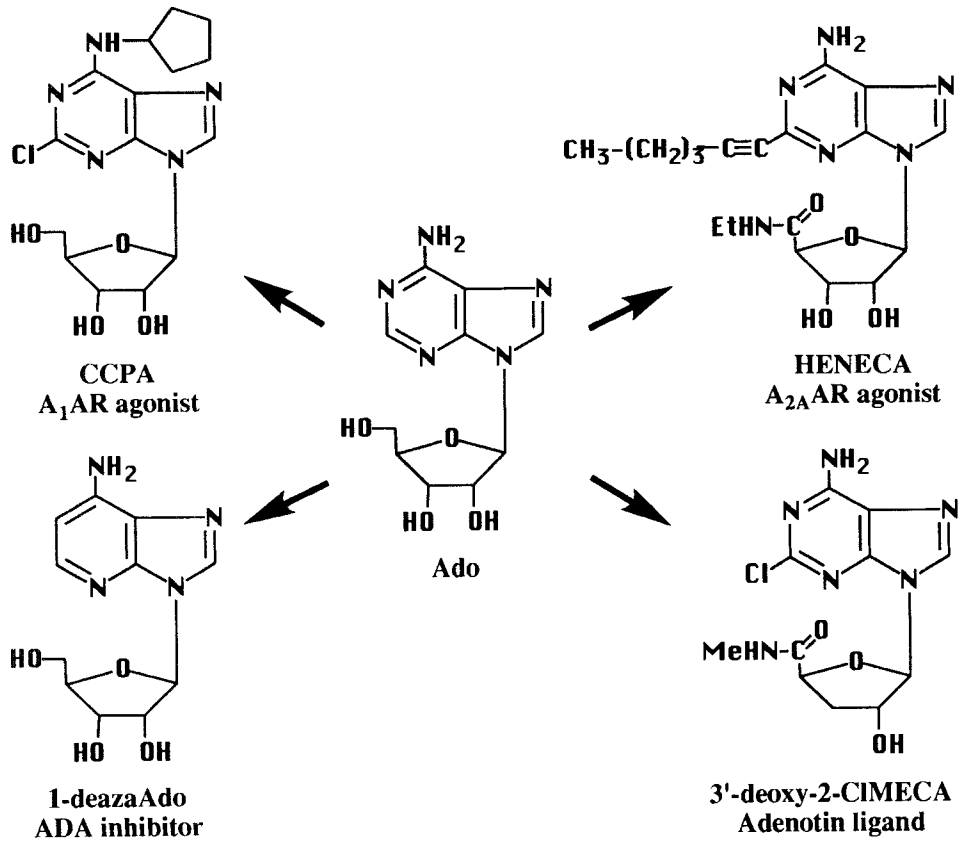


FIGURE 1. Some ligands for adenosine binding sites.

HENECA and A_{2A} adenosine receptor agonists

The efforts of medicinal chemists in the past 20 years led to the discovery of a variety of selective agonists for the different adenosine receptor subtypes. A common figure of such agonists is their structural relation with adenosine.

The potent and selective agonists for the A_{2A} receptors obtained in the last years were designed on the basis of two observations: a) adenosine-5'-N-ethyluronamide (NECA) has affinity in the low nanomolar range for both A_{2A} and A_1 receptors,[6] and is a potent tool in several pharmacological models b) introduction on C-2 of adenosine of a variety of substituents increased A_{2A} affinity and selectivity.

On these bases , we have reported the synthesis of the 2-hexynyl derivative of NECA, identified as HENECA, which showed high affinity at A_{2A} adenosine receptors and good A_{2A} vs A_1 selectivity.[7] HENECA displayed also inhibitory activity on platelet aggregation

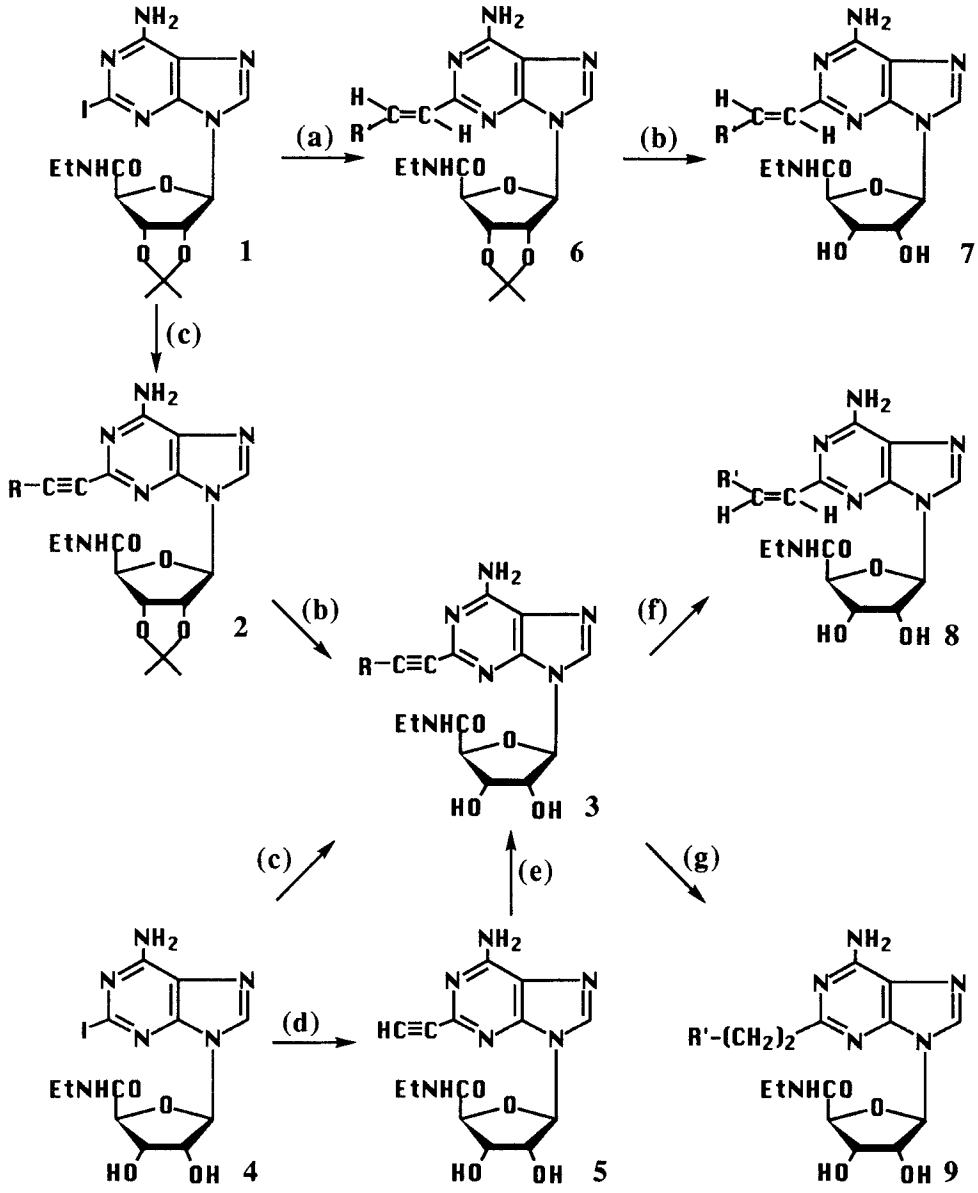
higher than NECA itself,[8] and has been extensively characterized using a variety of experimental models.[9,10]

The therapeutic potential of HENECA for the treatment of cardiovascular diseases prompted us to synthesize a number of new 2-alkynyl and cycloalkynyl derivatives of NECA bearing hydroxyl, amino, chloro, cyano and heterocyclic groups or substituted aromatic or heteroaromatic rings in the side chain.[11,12] Briefly, from the common intermediate, the 5'-carboxyl-2',3'-isopropyliden derivative, the two carboxamido derivatives **1** and **4** were obtained as starting material for the synthetic routes a-d depicted in Scheme 1.[13] Moreover, some alkenyl and alkyl derivatives of NECA were also synthesized, as classical modification of the triple bond, to better understand the conformational requirements of the receptor area interacting with the substituents in 2- and 5'-position.[14] In the case of the alkenyl derivatives, it is possible to design two geometric isomers: *cis* or Z (**8**), and *trans* or E (**7**). They were obtained by two different approaches illustrated in Scheme 1. The interaction of the new 2-alkynyl and 2-alkenyl derivatives of NECA with the adenosine receptors was evaluated using both radioligand binding technique and functional assays. In general, many compounds showed subnanomolar activity in the rat striatal binding experiments and some of them were very potent in inducing vasorelaxation without relevant effect on heart rate. In particular, the introduction of a 2-thiazolyl group led to a compound (**11**) with marked vasodilating activity among all the synthesized agonists (rat aorta, EC_{50} = 3.5 nM).

Partial reduction of the triple bond in 2-alkynyl derivatives of NECA led to compounds whose activity at A_{2A} receptor subtype was related to *Z-E* isomerism, the *E*-diastereomers being more potent and selective than the *Z* ones. Saturation of the side chain markedly reduced compounds affinity at adenosine receptors. The new nucleoside (*E*)-2-phenylpentenylNECA (**10**) exhibited both high A_{2A} receptor affinity (Ki = 3.5 nM) and almost 300-fold A_{2A} *vs* A_1 selectivity. Comparison between alkenyl derivatives of NECA and adenosine further demonstrated that the 5'-ethylcarboxamido group is critical for the A_{2A} affinity. These studies indicated also that the isomerism of the substituent in 2-position and the nature of the 5'-group in adenosine derivatives are critical to achieve high affinity and selectivity at the A_{2A} adenosine receptor subtype.[14]

All the synthesized compounds were also tested as inhibitors of platelet aggregation induced by ADP and the results are reported as potency ratio vs NECA.[11-13] Some compounds resulted more potent than HENECA itself as inhibitors of platelet aggregation.

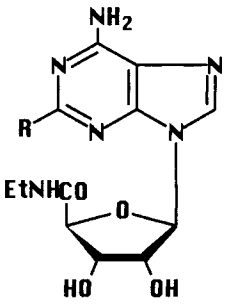
Among these alkynyl derivatives, phenylhydroxypropynylNECA (R,S-PHPNECA) behaved as a very potent agonist at A_1 and A_{2A} receptor subtypes, with a Ki of 2.5 nM and 0.9 nM, respectively. Furthermore, it showed an inhibitory activity on platelet aggregation, induced by ADP, about 16 fold higher than NECA, being the most potent nucleoside inhibitor of platelet aggregation reported so far.

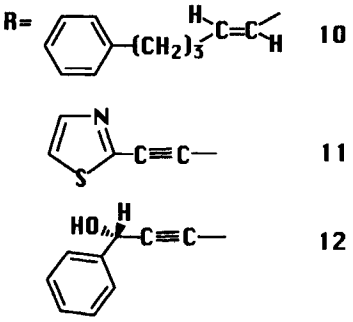


(a) Alkenyl catacholeborane derivatives, CH_3CN, tetrakis, and K_3CO_3 at 90°C. (b) CF_3COOH or 50% HCOOH. (c) Alkynes, CH_3CN or DMF, $(Ph_3P)_2PdCl_2$, CuI, and Et_3N. (d) 1) Trimethylsylylacetilene, CH_3CN or DMF, $(Ph_3P)_2PdCl_2$, CuI, and Et_3N; 2) KOH. (e) Aromatic halide (Br or I), DMF, $(Ph_3P)_2PdCl_2$, CuI, and Et_3N. (f) H_2 18 psi, Lindlar catalyst. (g) H_2 40 psi, 10% Pd/C (see Ref. 11-13).

SCHEME 1

TABLE 1. Pharmacological activity of selected NECA derivatives.





Compds	Binding assays Ki (nM)			Functional activity EC50 (nM)		Antiaggr. act. IC50 (nM)
	Rat brain A_1	Rat striatum A_{2A}	Selectivity A_1/A_{2A}	Ratatria A_1	Rat aorta A_{2A}	Rabbit platelets A_{2A}
HENECA	130	2.2	59	>10 μM	596	67
10	1017	3.5	291	>10 μM	576	1400
11	85.4	41.3	2.1	7160	28.7	450
12	4.0	0.5	8	52.0	29.9	6

For experimental details see Ref. 11-13

Since this compound bears a chiral carbon in the side chain, the enantiomeric resolution was undertaken to assess the enantioselectivity of A_{2A} adenosine receptors.[15] The S-diastereomer **12** proved to be more potent than the R-diastereomer, and than the mixture as well, in all model systems. However, the most important achievement is that the platelet receptor clearly discriminates between the two compounds since R-PHPNECA is equiactive with NECA, whereas the S-diastereomer shows an antiaggregatory potency about 40-fold that of NECA, i.e. about 6 nM, resulting the most potent inhibitor so far known in the adenosine agonist derivatives.

CCPA

A potent and selective agonist for the A_1 receptor was designed on the basis that the contemporary presence of a chlorine in 2-position and a cyclopentyl substituent on the N-6 amino group increased both A_1 affinity and selectivity.[16] This observation, related to 1-deazaadenosine derivatives, prompted us to synthesize the 2-chloro derivative of CPA,

namely CCPA.[17] This compound proved to be both more active and A_1-selective than the reference compound, showing A_1 affinity in the subnanomolar range and a selectivity three fold that of CPA. A detailed pharmacological profile of CCPA in binding, functional, and *in vivo* models has been recently reported by Monopoli et al.[18] CCPA is now available in the market also in the tritiated form, obtained by catalytic reduction in a tritium atmosphere of the corresponding cyclopentenyl derivative.[19]

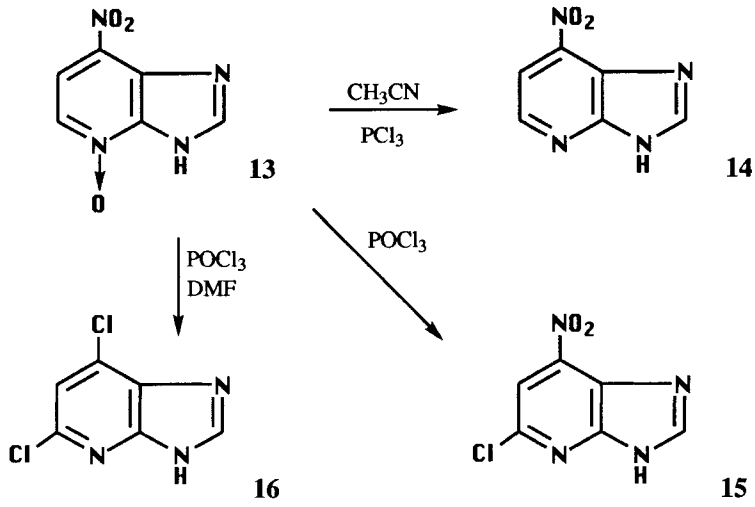
3'-Deoxy-2ClMECA

Studies performed with the non selective radioligand [^{3}H]NECA have recently demonstrated that in human platelet membranes, in addition to the A_{2A} receptor subtype, a non-receptor protein called "adenotin" is present.[20-22] Its identity or striking amino-terminal homology with stress-protein sequences and its high intracellular concentration (around 1% of protein in human placenta) suggest that adenotin could play an important role in the regulation of cellular functions like cell proliferation and tumor growth.[23] However, the specific role of adenotin must be determinated, yet. Binding studies, performed with typical adenosine ligands, indicated that Ado, N^6-substituted A_1 adenosine agonists and xanthine antagonists possess very low affinity for this protein.[23] 2-Chloroadenosine-5'-N-methyluronamide (ClMECA) proved to be the most active ligand so far known (Ki = 24 nM). However, it is an unselective ligands, possessing high affinity for both A_1 and A_{2A} adenosine receptors. In an attempt to identify a more potent and selective adenotin ligand we have undertaken the synthesis of 2'- and 3'-deoxy derivative of 2-ClMECA, on the basis that removing of hydroxyl groups from the sugar moiety of adenosine derivatives, while increases binding activity on adenotin, greatly compromises adenosine receptor affinity. Preliminary results of competition experiments for [^{3}H]NECA binding showed that 3'-deoxy-2-ClMECA (Figure 1) possesses very high affinity for adenotin (Ki = 13.7 nM) combined with a very good selectivity toward adenosine receptor subtypes, resulting in the most potent and selective adenotin ligand so far known.[24]

1-DeazaAdo derivatives

In our effort to synthesize deaza nucleosides endowed with pharmacological activity, we selected 1-deazapurine derivatives since 1-deazaadenosine (1-deazaAdo, Figure 1) has been shown to possess cytotoxic activity,[25] to inhibit adenosine deaminase[26] and platelet aggregation,[16] and to act as an agonist of adenosine receptors.[27]

We have already reported the synthesis of 7-nitro-3H-imidazo[4,5-b]pyridine (**14**),[25] 5-chloro-7-nitro-3H-imidazo[4,5-b]pyridine (**15**),[27] and 5,7-dichloro-3H-imidazo[4,5-b]pyridine (**16**),[27] obtained starting from the common intermediate 7-nitro-3H-imidazo[4,5-b]pyridine-4-oxide (Scheme 2).[28]

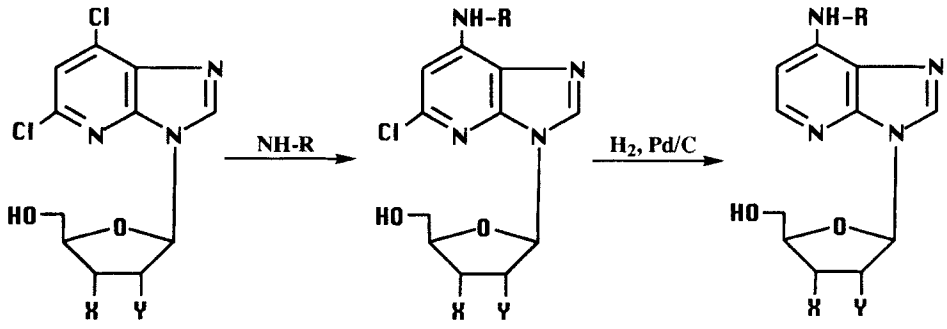


SCHEME 2

The coupling of **2** and **3** with ribose and 2-deoxyribose derivatives gave, after working up, 6-amino-[25] and 6-hydroxylamino-1-deazapurine nucleosides.[29,30] Since 5,7-dichloro-3H-imidazo[4,5-b]pyridine (**16**) is a more versatile base, it has been coupled with a variety of sugar moieties (**17-20**) and transformed in a series of 7-alkyl(aryl)amino derivatives by reacting with the suitable amines (**21-28**, Scheme 3).[31,32]

These compounds were evaluated for their *in vitro* activity against human immunodeficiency virus type-1 (HIV-1) and herpes simplex virus type-1 (HSV-1).

In addition they were tested for their ability to inhibit adenosine deaminase (ADA) from calf intestine.[32] While the parent compounds 1-deazaadenosine (**25a**), 2'-deoxy-1-deazaadenosine (**26a**) and 2',3'-dideoxy-1-deazaadenosine (**27a**) and the corresponding 2-chloro derivatives were inactive, nucleosides bearing cycloalkyl substituents on N^6 exhibited moderate to good anti-HIV-1 activity, compared to 2',3'-dideoxyadenosine (ddA), with the degree and pattern of improvement depending on the structure of the sugar moiety. In general, 2'-deoxy- and 2',3'-dideoxy derivatives were more potent compounds than the corresponding ribose nucleosides. Compounds bearing a 6-cycloheptyl or cyclooctylamine were the most active in every series. The presence of a chloro group in 2-position improved both activity and therapeutic index in every series, the most active compound being 2'-deoxy-2-chloro-N^6-cycloheptyl-1-deazaadenosine (**22g**, $ED_{50} = 0.2$ μM). On the other hand, most of these derivatives were inactive as anti-HSV-1 agents, showing a high degree of virus-selectivity. The 1-deazaadenine derivatives are not



17-20 **21-24 (a-h)** **25-28 (a-h)**

R= (a) H, (b) CH_3, (c) cC_3H_5, (d) cC_4H_7, (e) cC_5H_9, (f) cC_6H_{11}, (g) cC_7H_{13}, (h) cC_8H_{15}

X = OH, Y = OH; Ribose (17, 21, 25)

X = OH, Y = H; 2'-Deoxyribose (18, 22, 26)

X = H, Y = H; 2',3'-Dideoxyribose (19, 23, 27)

X = H, Y = OH; 3'-Deoxyribose (20, 24, 28)

SCHEME 3

substrates of adenosine deaminase: besides, some of them are good inhibitors of the enzyme. 1-Deaza-2'-deoxyadenosine (**26a**) is the most potent in the series (K_i = 0.19 mM) and resulted to be more active than 1-deazaadenosine itself (K_i = 0.66 mM). However, the ADA inhibitory activity does not account for the antiviral potency since increased lipophilicity and steric hindrance of substituents resulted in derivatives much less active than the parent compounds. According to the fact that introduction of a chloro group in position 2 of substrates made the compounds more resistant to ADA,[33] the presence of a chloro group in the same position of our compounds produced a decrease in ADA inhibitory activity. This is in agreement with our hypothesis that 1-deazaadenosine derivatives interact with the enzyme directly on the catalytic site, since the presence of a bulky, hydrophobic substituent on the exocyclic nitrogen is detrimental for the hydrogen bonding of the molecules.[26b]

More recently the synthesis of the 3'-deoxy derivatives of 1-deazaadenosine has been achieved in good yield by coupling compound **16** with 1,2-di-O-acetyl-5-O-benzoyl-3-deoxy-D-ribofuranose[34] in the presence of $SnCl_4$ (**20**).[35] The pharmacological activities of the new nucleosides are under investigation.

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