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Purine and deazapurine nucleosides: synthetic approaches, molecular modelling and biological activity

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Abstract

A number of ligands for the adenosine binding sites has been obtained by using nucleoside convergent and divergent synthesis. Most of our nucleosides have been synthesized by coupling 2,6-dichloropurine (**1**), 2,6-dichloro-1-deazapurine (**2**), 2,6-dichloro-3-deazapurine (**3**) with ribose, 2- and 3-deoxyribose and 2,3-dideoxyribose derivatives. The use of these versatile synthons allowed the introduction of various substituents in 2- and/or 6-positions. The glycosylation site and the anomeric configuration of the obtained nucleosides were assigned on the basis of spectroscopic studies and confirmed by molecular models. A series of potent adenosine receptor ligands has been obtained by using divergent approaches, mostly starting from guanosine. Substitutions in 2, 6, 8, and 5′ position of adenosine molecule led to ligands selective for the different adenosine receptor subtypes. Furthermore, we investigated the molecular bases of the different behavior of 2- and 8-alkynyl adenosines, by means of NMR experiments and molecular modeling studies. With docking experiments, we demonstrated that the two class of molecules should have different binding modes that explain their different degree of affinity and the shift of their activity from agonistic (2-substituted derivatives) to antagonistic (8-substituted derivatives).

Keywords: Adenosine receptor ligands; Adenosine receptor molecular modeling; Glycosylation reactions; Nucleoside synthesis

1. Introduction

Purine and deazapurine nucleosides are of great interest both from chemical and pharmacological point of view. In particular, compounds structurally related to the intracellular modulator adenosine have been shown to possess biological activity as adenosine receptor ligands [1], antiviral and antitumor agents [2], and enzyme inhibitors [3].

Our group has been involved since many years in this research field and we have extensively described coupling of substituted purines and deazapurines with a variety of sugars. An attempt to resume such synthetic work is shown in Fig. 1.

2. Convergent syntheses

Most of our nucleosides have been synthesized by coupling 2,6-dichloropurine (**1**), 2,6-dichloro-1-deazapurine (**2**), 2,6-dichloro-3-deazapurine (**3**) with ribose, 2- and 3-deoxyribose and 2,3-dideoxyribose derivatives. The use of these versatile synthons allowed the introduction of various substituents in 2- and/or 6-positions: in particular, in the case of the less reactive 1- and 3-deazanucleosides, nucleophilic substitutions in 6-position can be achieved under milder conditions when a chlorine atom is present in 2-position [4–9].

For the synthesis of hydroxylamine derivatives of purines and 1-deazapurines two additional synthons have been used, 6-nitro-1-deazapurine (7-nitro-3*H*-imidazo[4,5-*b*]pyridine, **4**) and 2-chloro-6-nitro-1-deazapurine (5-chloro-7-nitro-3*H*-imidazo[4,5-*b*]pyridine, **5**), which were prepared starting from the common intermediate 6-nitro-1-deazapurine-3-oxide (7-nitro-3*H*-imidazo[4,5-*b*]pyridine-4-oxide) [10].

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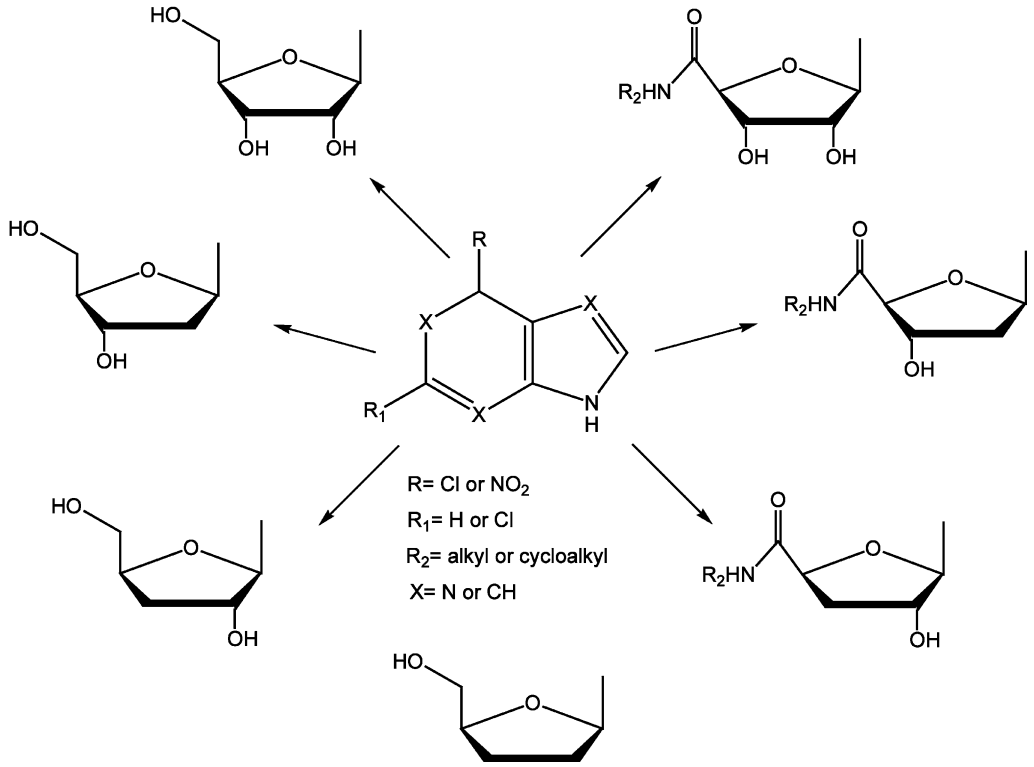
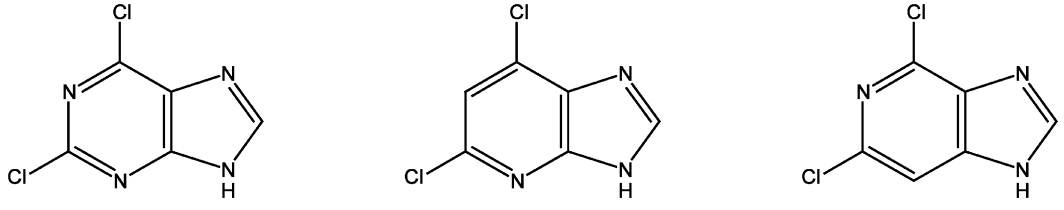


Fig. 1. Coupling of substituted purines and deazapurines with various sugars.

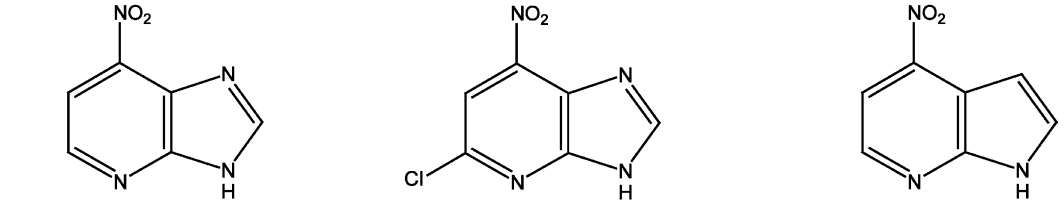
For the synthesis of 1,7-dideazapurine derivatives 6-nitro-1,7-dideazapurine (4-nitro-1*H*-pyrrolo [2,3-*b*]pyridine, **6**) was utilized [11]. All these synthons are reported in Fig. 2.

Depending upon coupling conditions as well as base and sugar structure and reactivity, different anomeric and isomeric mixtures have been obtained. Extensive studies, utilizing chemical and physical methods, have been performed to assign the correct configuration to the resulting nucleosides.

We report here on the coupling of 2,6-dichloropurine and 1- and 3-deazapurine with 3-deoxyribose, which has been recently and extensively investigated in our laboratory. These coupling reactions have been carried out both by fusion method and by acidic catalysis. In the fusion method, reported below, coupling was performed



1 2,6-dichloro purine **2** 2,6-dichloro-1-deazapurine **3** 2,6-dichloro-3-deazapurine



4 6-nitro-1-deazapurine **5** 2-chloro-6-nitro-1-deazapurine **6** 6-nitro-1,7-dideazapurine

Fig. 2. Substituted purines and deazapurines.

in the presence of a catalytic amount of *p*-toluensulphonic acid at 160 °C in vacuo for 10 min.

Coupling of 2,6-dichloropurine with 1,2-di-*O*-diacetyl-5-*O*-benzoyl-3-deoxy-β-D-ribofuranose (**7**) gave a mixture of beta (**8**) and alfa (**9**) anomers in one to one ratio and in a 65% total yield. Deblocking with liquid ammonia at room temperature both removed the protections and introduced the amino group in 6-position to yield compounds **10** and **11**. Other amines can be introduced in 6 position at this stage by using the suitable reagent. However, it no possible to obtain deprotection without substitution in 6-position (Scheme 1) [12].

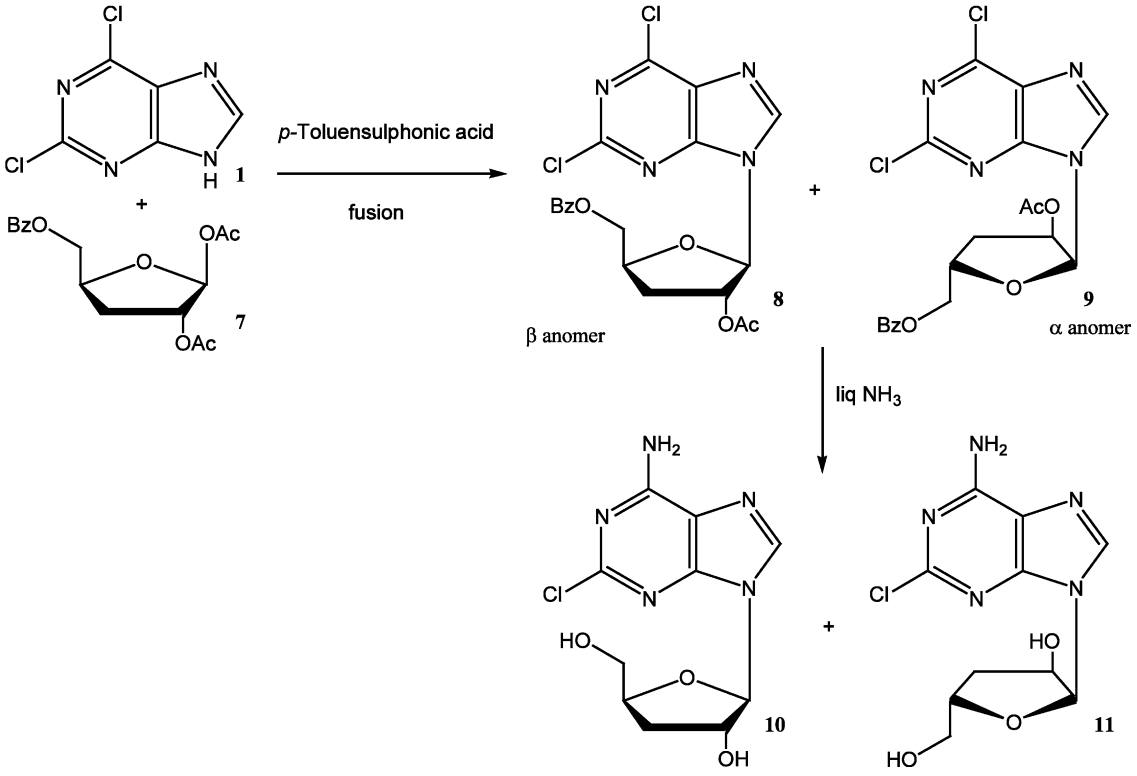
Coupling of 2,6-dichloro-1-deazapurine (5,7-dichloro-3*H*-imidazo[4,5-*b*]pyridine (**2**) with the same sugar **7** gave again a mixture of beta (**12**) and alfa (**13**) anomers in 2 to 1 ratio and in a total yield of 81%. In this case, deblocking with methanolic ammonia at room temperature led to the 2,6-dichloro deblocked nucleosides **14** and **15** in 85% total yield. In fact, differently from purines, with 1-deazapurines it is possible to obtain deprotection without substitution in 6-position (Scheme 2) [8].

Coupling of 2,6-dichloro-3-deazapurine (4,6-dichloro-1*H*-imidazo[4,5-*c*]pyridine, **3**) with 1,2-di-*O*-diacetyl-5-*O*-benzoyl-3-deoxy-β-D-ribofuranose (**7**) gave a mixture of four nucleosides in a 90% total yield. By reacting this mixture with methanolic ammonia for 24 h at room temperature the corresponding deprotected derivatives were obtained (51, 23, 3 and 13% yield, respectively) after purification and the structure reported in Scheme 3 was assigned to compounds **16**, **17**, **18** and **19**. Accordingly, the four nucleosides show the same glycosylation site and correspond to beta and alfa ribofuranose derivatives and beta and alfa arabinofuranose derivatives, respectively [7].

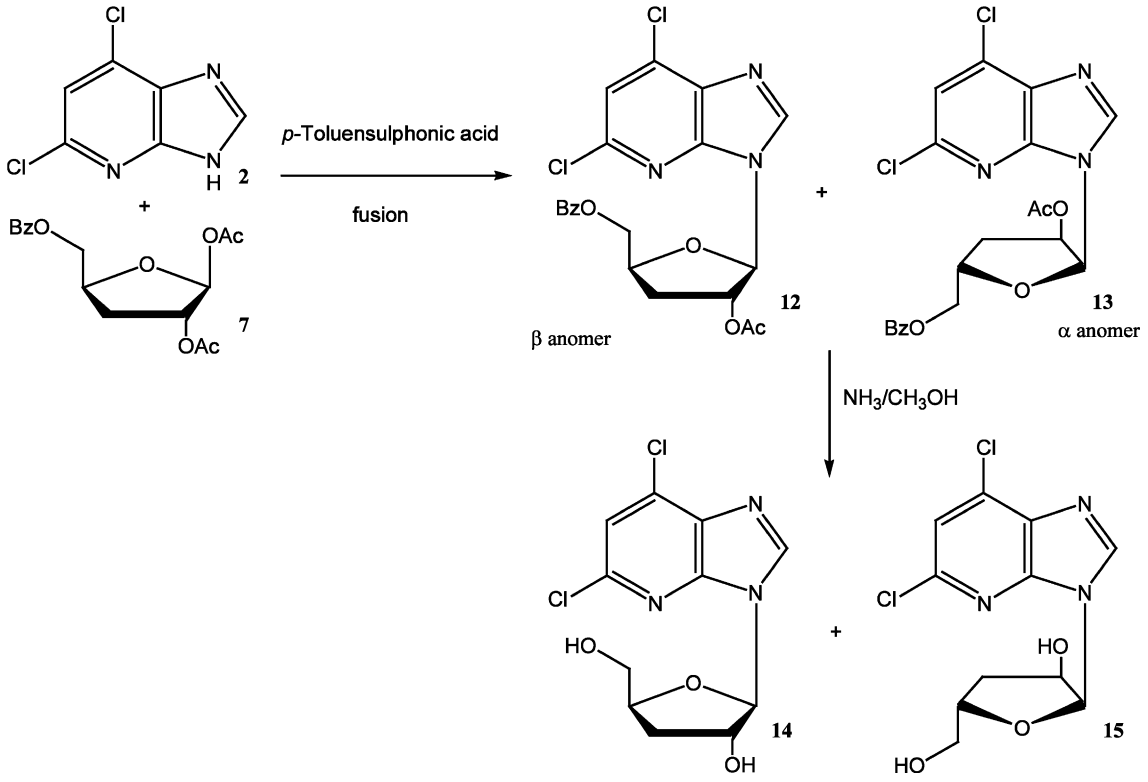
The glycosylation site and the anomeric configuration of these nucleosides were assigned on the basis of spectroscopic studies. The UV spectra of the four nucleosides are practically identical showing a maximum of absorption at 259 nm with an extinction coefficient of about 13 000; these findings suggested that the glycosylation site was the same for all the obtained compounds.

^{1}H NMR spectra and NOE studies, performed by irradiating different protons of the sugars, demonstrated unequivocally the structures of the four nucleosides. In fact, studies of 1D ^{1}H NOE differential spectroscopy established unequivocally N(9) as glycosylation site in the case of all compounds **16**–**19** (Table 1a). Accordingly, saturation of H–C(1′) resulted in a NOE at the H–C(8) and H–C(3) signal. These data excluded without any doubt the presence of a possible glycosylation occurred at N(7). In this case, in fact, no NOE effect at the H–C(3) signal would have been found.

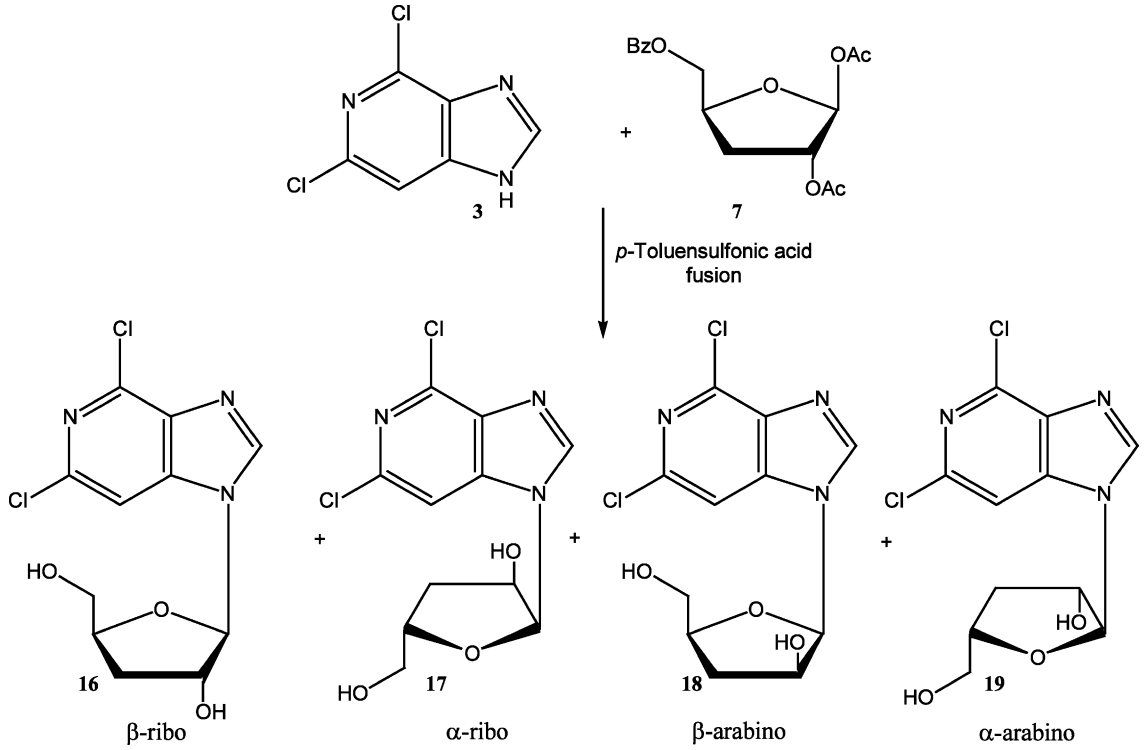
The epimerization at C(2′) of nucleosides **18** and **19** has been demonstrated by the NOE observed at the H–C(3′b) signal upon saturation of both H–C(2′) and H–



Scheme 1.



Scheme 2.



Scheme 3.

Table 1

Comp.	Irradiated proton	Observed NOE (%)
(a) NOE (%) data of compounds ***16, 17, 18*** *and* ***19*** *in* (D_6–*DMSO*) *at 23°*		
16	H–C(1′)	H–C(2) (4.6), H–C(7) (8.9), H–C(2′+4′) (6.0)
17	H–C(1′)	H–C(2) (4.2), H–C(7) (6.7), H–C(2′) (6.8), H–C(3′a) (1.0)
18	H–C(1′)	H–C(2) (3.2), H–C(7) (4.0), H–C(2′) (3.0)
	H–C(2′)	H–C(1′) (4.4), H–C(3′b) (1.8)
	H–C(4′)	H–C(3′b) (2.8)
19	H–C(1′)	H–C(2) (4.2), H–C(7) (6.5), H–C(2′) (2.0), H–C(3′a) (0.9)
	H–C(2′)	H–C(2) (3.0), H–C(7) (3.0), H–C(1′) (2.1), H–C(3′b) (3.1)
	H–C(4′)	H–C(2) (1.7), H–C(7) (1.4), H–C(3′b) (2.9)

Comp.	**16**		**17**		**18**		**19**	
(b) Distances of protons from H–C(1′) and relative NOE effect upon H–C(1′) saturation								
	Å	NOE	Å	NOE	Å	NOE	Ang	NOE
H–C(2′)	3.049	6.0 [a]	2.359	6.8	2.351	3	3.057	2
H–C(3′a)	4.152		3.453	1	4.169		3.436	0.9
H–C(3′b)	3.535		4.153		3.809		4.167	
H–C(4′)	3.486	6.0 [a]	3.856		3.627		3.844	
H–C(2) (*anti*)	3.995		3.973		3.979		4.006	
H–C(7)	2.320	8.9	2.337	6.7	2.308	4	2.281	6.5
H–C(2) (*syn*)	2.611	4.6	2.589	4.2	2.581	3.2	2.634	4.2
H–C(7)	4.206		4.222		4.212		4.181	

[a] H–C(2′)+H–C(4′).

C(4′). This result confirmed that the three protons are located on the same side of the tetrahydrofuryl ring.

Irradiation of H–C(1′) of compound **19** gave NOE at the H–C(2′) and the H–C(3′a) signals, proving the alfa configuration. On the other hand, saturation of H–C(1′) of compound **18**, resulted in a NOE at the H–C(2′) signal but not at the one of H–C(4′). The lack of this effect, even though is rather strange for a beta nucleoside, could be explained by the distortion that the arabinose introduces in the nucleoside because of the position of the OH–C(2′).

On the bases of 1D ^{1}H NOE obtained, molecular models were constructed by utilizing the program HYPERCHEM [13]. The structures were optimized by using the semiempirical method PM3 with the minimization algorithm Eigenvector Following until a gradient RMS of 10^{-3} kcal/Å mol was reached. As shown in Table 1b, the distances from the H–C(1′) calculated for the protons of the four nucleosides are in very good agreement with the experimental data.

The other convergent approach was the coupling reaction carried out in acetonitrile and using stannic chloride or TMS triflate as acidic catalyst. In this case, only the beta anomer was obtained with all the three bases.

Couplings of the same bases with (cyclo)alkyl halides have been also carried out, aimed at obtaining adenosine receptor antagonists [14,15] and enzyme inhibitors [3].

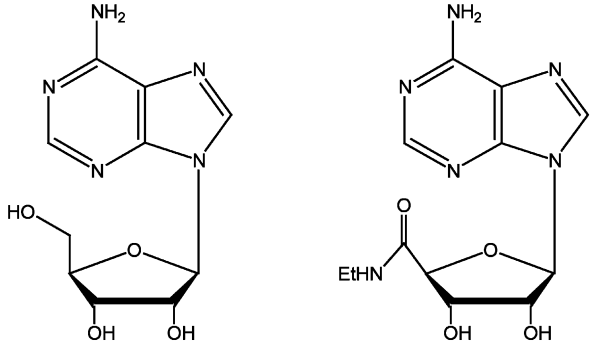


Fig. 3. Adenosine and NECA.

3. Synthesis and biological evaluation of adenosine receptor agonists obtained by using divergent approaches

Other series of adenosine receptor ligands have been obtained by using divergent approaches. Alkynyl chains were introduced in 2- and 8-position of adenosine (**20**) and 5′-*N*-ethylcarboxamidoadenosine (NECA, **21**) by multi-step charts starting from guanosine (**22**) or 8-bromoadenosine (**23**), respectively [16] Fig. 3.

Combination with substitutions in 6-position yielded agonists potent and selective for the various adenosine receptor subtypes. Adenosine is in fact a signaling molecule that, among its various biological functions, can interact with four specific membrane receptors belonging to the purinergic P1 family and named A_1, A_{2A}, A_{2B} and A_3 [17]. All these subtypes belong to the super-family of 7 *trans*-membrane domains G-protein coupled receptors (7TMs GPCR) and are classified by

the GPCR database [18] in the family of rhodopsine like receptors (Class A of GPCR). Among human adenosine receptors, the A_3 subtype was the last one to be identified, and, unlike what happened for A_1, A_{2A} and A_{2B}, it was discovered by means of molecular biology techniques followed by pharmacological experiments.

The biological functions of the A_3 subtype are still not very well understood. This is mainly due to the lack of truly selective ligands for in vivo studies and to the poor structural characterization of the receptor itself. In fact just few mutagenesis studies on this subtype have been carried out [19–21].

There are reports that agonists acting via the adenosine A_3 receptor have cardioprotective effects [22,23] although it was proven that the A_3 receptor serves a different cardioprotective function as compared with the adenosine A_1 receptor [24]. Chronic administration of adenosine A_3 receptor agonists has also been shown to have protective actions in the brain, in contrast to acute high-dose administration, which can cause toxicity [25,26].

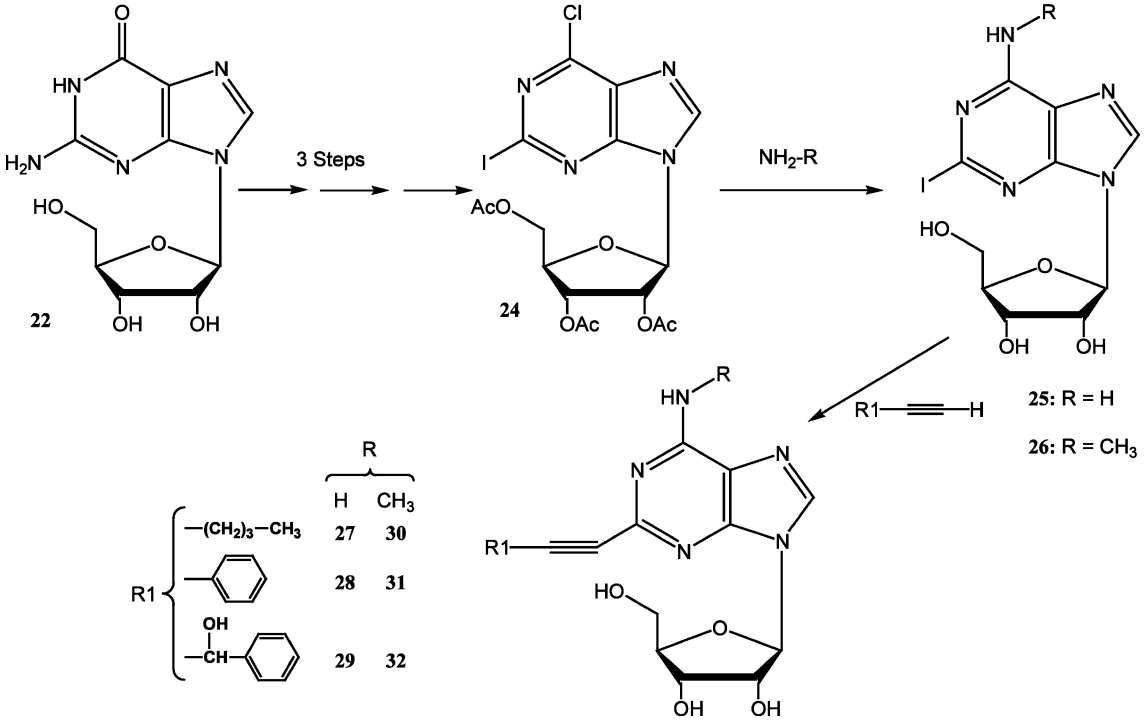
In recent years we demonstrated that C-2-substituted NECA are potent and selective ligands for adenosine receptor subtypes [27,28]. In particular compounds bearing a hexynyl, phenylethynyl, and phenylhydroxypropynyl chains at the 2-position of NECA possess high affinity for A_3 receptors combined, in same cases, with good selectivity [15]. In order to investigate the role of the carboxamido group at the 5′-position and to simplify the structure of these molecules, adenosine derivatives bearing the above mentioned alkynyl chains at the C-2-position were synthesized [29].

Starting from commercially available guanosine very versatile synthons functionalized in three different positions can be obtained. In Scheme 4 the approach to obtain 2,6-disubstituted adenosines is reported. Guanosine (**22**) is converted in three steps in 6-chloro-2-iodo acetylated adenosine (**24**) [30], from which the two substitutions can be carried out.

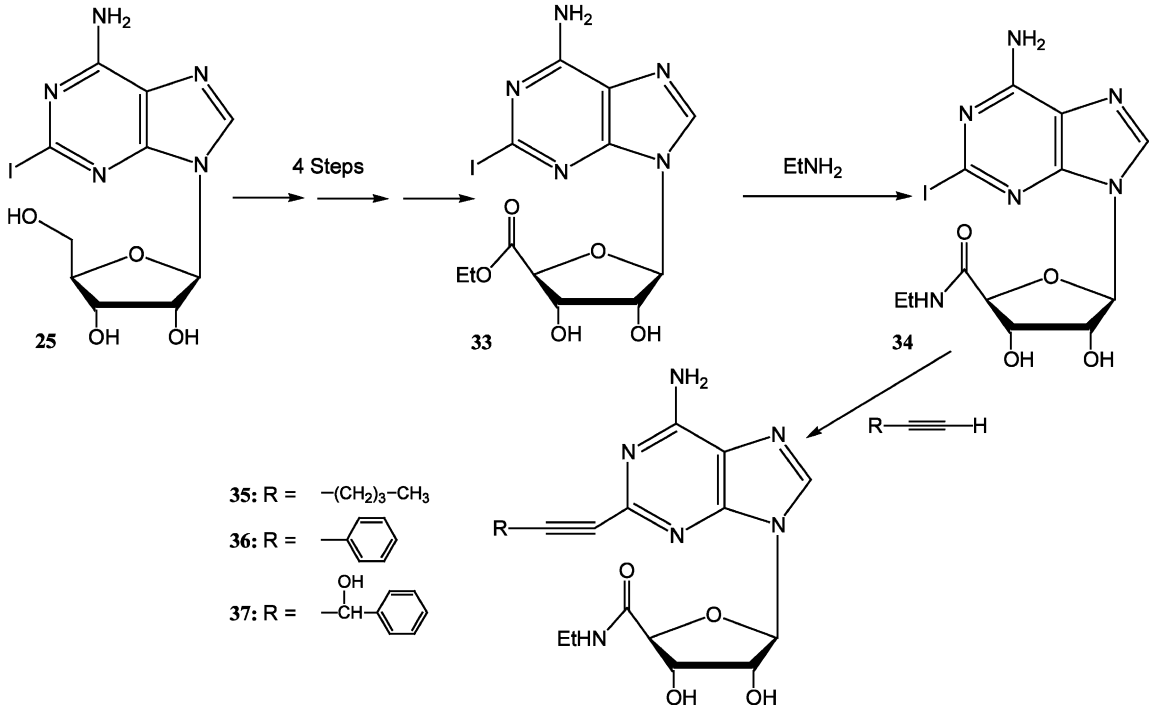
Other disubstituted adenosines can be obtained according to the procedure depicted in Scheme 5. In this case the starting material 2-iodoadenosine (**25**) comes from the previous method and is transformed in four steps in 2-iodo-5′-ethylesteradenosine **33**, from which 2-alkynylNECAs **35**–**37** have been obtained [27].

Trisubstituted adenosines can be obtained by using 2-iodoNECA (**34**) as the starting material. Diazotation of the 6-amino group, followed by nucleophilic substitution of the 6-iodine atom gave compounds of general structure **39**, which can be additionally modified in 2-position by using the cross coupling reaction or nucleophylic substitutions to obtain compounds of general formula **40** (Scheme 6) [31,32].

Recently, the four human receptor subtypes have been transfected and overexpressed on CHO cells. Whereas radioligand binding studies have been utilized for A_1, A_{2A} and A_3 receptors, the relative potencies of agonists for the A_{2B} adenosine receptor have been tested by measuring the activity of receptor-stimulated adenylyl cyclase [33,34]. We are currently utilizing these tranfected CHO cells for the development of new ligands and the affinity and potency at the four subtypes of the



Scheme 4.

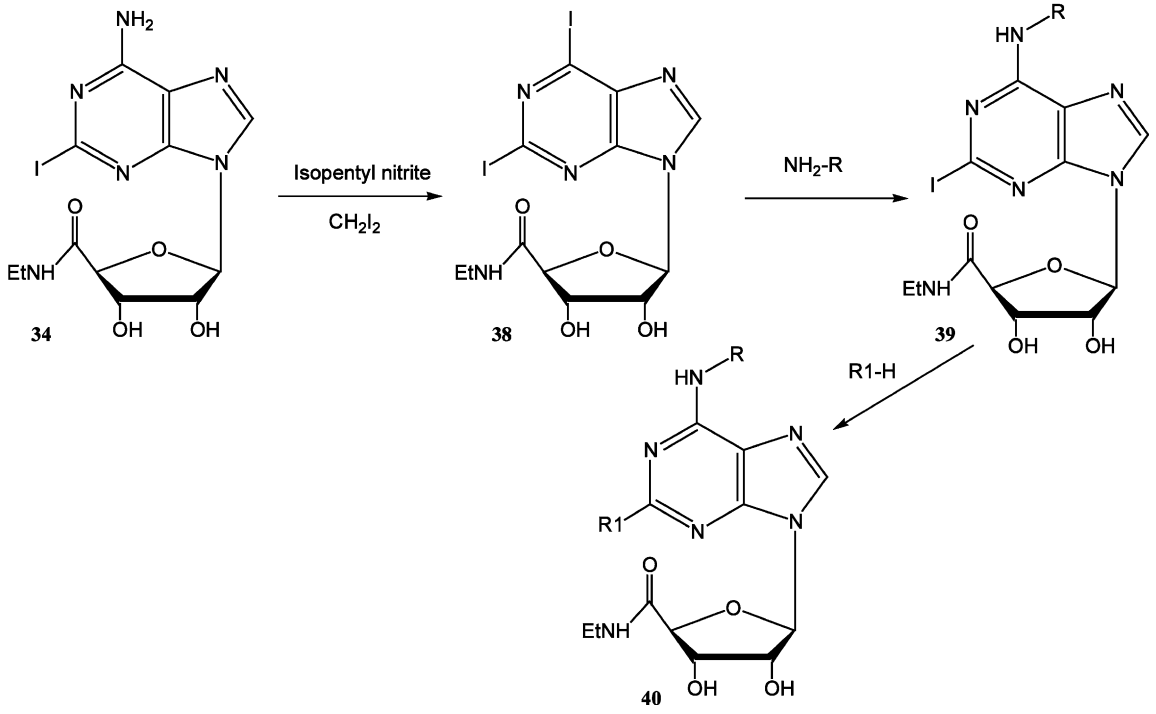


Scheme 5.

selected 2-alkynyl derivatives of adenosine and NECA are reported in Table 2.

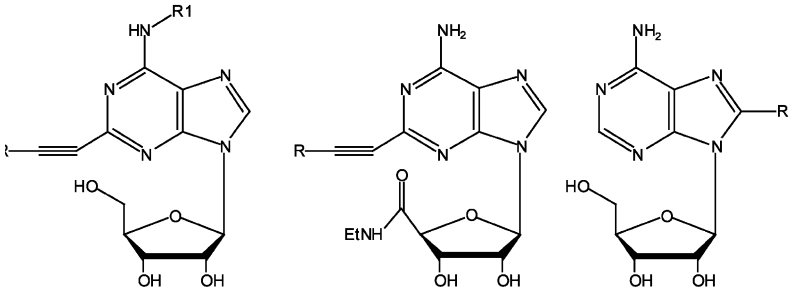
From these data it is possible to note that the introduction of alkynyl chains on the adenosine structure led to compounds with high affinity and different degree of selectivity on A_1, A_{2A}, and A_3 subtypes. In particular, compound **28**, bearing a phenylethynyl group, presents high affinity and selectivity for A_3 receptors, whereas the 2-phenylhydroxypropynyl derivative **29** is more potent but less selective for all this three subtypes.

Modification of the sugar by introducing a *N*-ethylcarboxyamido group enhanced the affinity of all compounds and the selectivity of the phenethynyl derivative **36** at the A_3 subtype.



Scheme 6.

Table 2
Adenosine derivatives affinities in radioligand binding assays at human A_1, A_{2A} and A_3 receptors, and in adenylyl cyclase assays at human A_{2B} subtype



Comp.	R	R1	K_i or EC_{50} (nM)			
			K_i (A_1) [a]	K_I (A_{2A}) [b]	EC_{50} (A_{2B}) [c]	K_I (A_3) [d]
27 2-HEAdo	CH_3–$(CH_2)_3$	H	18	5.7	100 000	4.7
28 2-PEAdo	C_6H_5	H	391	363	> 100 000	16
29 2-PHPAdo	C_6H_5–CH(OH)	H	0.67	7.0	2400	3.3
30	CH_3–$(CH_2)_3$	CH_3	327	1230	100 000	1.1
31	C_6H_5	CH_3	1690	8530	100 000	3.4
32	C_6H_5–CH(OH)	CH_3	8.4	273	2400	0.76
35 HENECA			60	6.4	6100	2.4
36 PENECA			560	620	> 100 000	6.2
37 PHPNECA			2.7	3.1	1100	0.42
41 8-HEAdo	CH_3–$(CH_2)_3$–≡–		> 100 000	> 100 000	> 100 000	650
42 8-PEAdo	C_6H_5–≡–		> 100 000	> 100 000	> 100 000	790
43 8-PKPAdo	C_6H_5–C(O)–=–		> 100 000	46 600	> 100 000	9830

[a] Displacement of specific [^{3}H]CCPA binding in CHO cells, stably transfected with human recombinant A_1 adenosine receptor, expressed as K_i (nM).
[b] Displacement of specific [^{3}H]NECA binding in CHO cells, stably transfected with human recombinant A_{2A} adenosine receptor, expressed as K_i (nM).
[c] Measurement of receptor-stimulated adenylyl cyclase activity in CHO cells, stably transfected with human recombinant A_{2B} adenosine receptor, expressed as EC_{50} (nM).
[d] Displacement of specific [^{3}H]NECA binding in CHO cells, stably transfected with human recombinant A_3 adenosine receptor, expressed as K_i (nM).

But the most relevant results have been obtained by introducing on the adenosine structure of a small substituent on the 6-amino group. In fact in this case both selectivity and affinity of the phenylethynyl derivative **31** for the A_3 subtype has been improved. On this basis, an application of the trisubstitution chemical approach, described in Scheme 6, can be depicted. In fact, the data reported in Table 2 demonstrated that the 2-alkynyl derivatives of adenosine possess high affinity for all AdoR subtypes.

The presence of a 5′-ethylcarboxamido group enhanced A_3 affinity of these molecules.

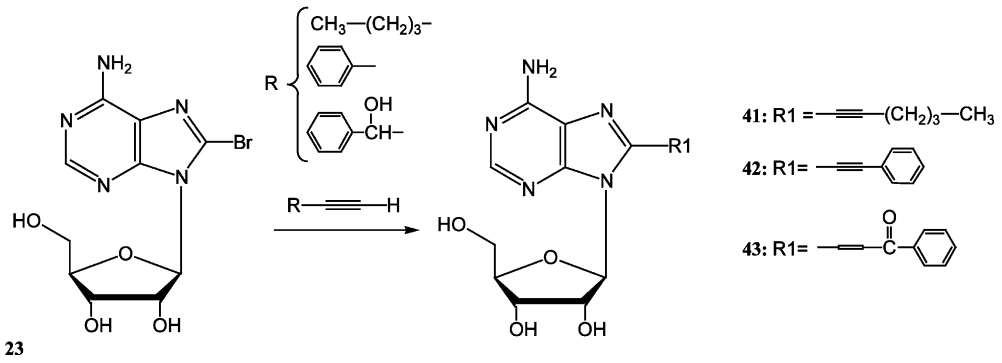
The presence of a N^6-methyl group in 2-alkynyl adenosines greatly improved A_3 selectivity.

Hence, combination of these three substitutions should be carried out to obtain molecules which should possess higher affinity and selectivity for the A_3 receptor subtype.

Furthermore, we wanted to investigate the effect of the same alkynyl chains in a different position of the purine ring, and so we moved them from the 2- to the 8-position of the molecule, also because this kind of substitution was not too much investigated in the past. Hence, 8-substituted adenosines **41**–**43** were synthesized by cross-coupling reaction starting from 8-Br-adenosine (Scheme 7) [35,36].

Unfortunately, the reaction with the phenylhydroxypropynyl chain did not provide the desired compound but a product deriving from the tautomeric rearrangement of the side-chain which ended up being a phenylketopropene (**43**).

The results of the binding studies carried out with the 8-substituted compounds (Table 2) show that the introduction of an alkynyl chain in C-8 position is very disadvantageous for the affinity and potency at A_1, A_{2A} and A_{2B} receptors, while is more tolerated by the A_3 subtype. The new 8-alkynyl derivatives, in fact, are endowed with an affinity for A_3 receptors in the high nanomolar range.



Scheme 7.

Furthermore, the maximal induction of guanosine 5′-(γ-thio)triphosphate ([^{35}S]GTPγS) binding to G proteins and the inhibition of NECA-stimulated binding, in membranes of CHO cells, which express the human A_3 receptor, were used to determine the intrinsic activity of these nucleosides at the human A_3 adenosine receptor. The results showed that these new adenosine derivatives are very selective ligands for the A_3 receptor subtype and behave as adenosine antagonists, since they do not stimulate basal [^{35}S]GTPγS binding, but inhibit NECA-stimulated binding, as shown in Fig. 4. This is the first report that adenosine derivatives, with unmodified ribose moiety, could behave as adenosine receptor antagonists.

4. NMR and molecular modelling studies

Since 2- and 8- alkynyl adenosines behave in a so different way we wanted to investigate their conformation about the glycosidic bond.

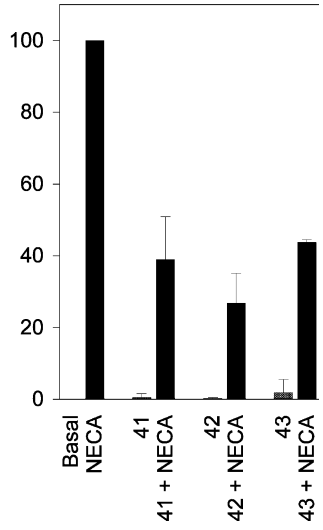


Fig. 4. Percentage of stimulation of [^{35}S]GTPγS binding (gray bars) and percentage of inhibition of NECA-stimulated [^{35}S]GTPγS binding (black bars) by compounds **41**–**43** in membranes of CHO cells which express the human A_3 receptor.

It is well known, in fact, that nucleosides are endowed with free rotation around the glycosidic bond and that they generally have two minima of energy, one in *syn* and the other in *anti* conformation. The conformational preference of these molecules, obviously, is affected by the surroundings, so different conformations can be found in solid state, in solution, in vacuo or upon binding to a protein.

It has been demonstrated by Bruns, on the bases of a detailed SAR analysis of adenosine receptor agonists that the binding of adenosine must occur in *anti* conformation [37] and this data was later confirmed by a number of mutagenesis and molecular modeling experiments.

On the other hand, Stolarski and Dudycz demonstrated that the chemical shift of H2′ in purine nucleosides can be used as an indicator of the sugar-base orientation. Typical Δ values for H2′ in DMSO, in fact, are 5.2 ppm for compounds restricted in *syn* conformation and 4.2 ppm for those in *anti* [38,39]. This big difference is explained by the influence that the nitrogen in 3 position can exerts on H2′ when the nucleoside is in *syn* conformation.

Since the *syn*–*anti* equilibrium is very rapid, the H2′ chemical shift of a given compound will have an average value determined by the statistical distribution of the various conformations. Adenosine, for example, shows an absolutely intermediate value of 4.62 ppm.

The 2-alkynyl derivatives **27**–**29** presented slightly lower chemical shifts, which indicate a moderate pre-

Table 3
H2′chemical shift of 2- and 8-substituted adenosines in DMSO solution

Comp.	H2′ chemical shift
Adenosine (**20**)	4.62
2-HEAdo (**27**)	4.57
2-PEAdo (**28**)	4.54
2-(*R*,*S*)-PHPAdo (**29**)	4.52
8-HEAdo (**41**)	5.04
8-PEAdo (**42**)	5.03
8-PKPAdo (**43**)	4.89

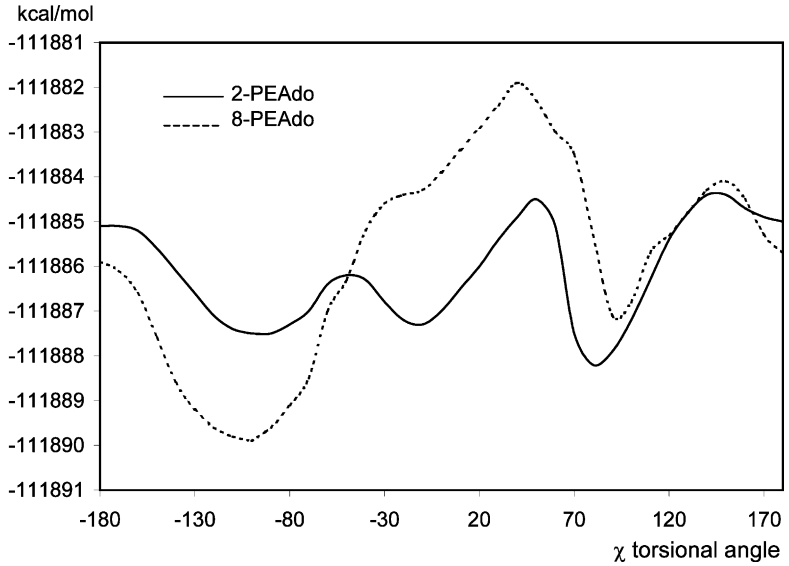


Fig. 5. Total energy (AM1) of 2- and 8-PEAdo in dependence of the O4′–C4′–C5′–O5′ (χ) torsional angle.

ference for the *anti* conformation. On the other hand, the 8-derivatives **41**–**43** showed a chemical shift of about 5 ppm, which reveals a clear preference for the *syn* conformation (Table 3).

After the NMR studies, some theoretical calculation utilizing the semi-empirical method AM1 was performed. The energetic differences, in kcal/mol, between the *syn* and the *anti* conformation of 2- and 8-alkynyl adenosine derivatives are reported in Fig. 5.

It is clear that, from the energy point of view, the 2-substituted compounds prefer the *anti* conformation, while the 8-substituted prefer the *syn* one. It is worth to note that the torsional angle found in *syn* conformations is always about -100°, and so the nitrogen in 3 position results to be very close to the H2′. Hence, the NMR and theoretical data are in very good agreement [40].

Furthermore, to investigate how this structural differences can affect the interactions with the adenosine receptors, we tried to get an insight into the molecular structure of the A_3 subtype and its binding site using molecular modeling techniques.

For this purpose, using the new crystal structure of bovine rhodopsine as template, after the alignment of the amino acid sequences, the model of the seven *trans*-membrane domains of the receptor with the homology modeling technique was constructed [41,42]. The agonist 2-phenylethynyladenosine (2-PEAdo, **28**) was docked in its *anti* conformation between the helices 3, 5, 6 and 7, as showed in the Fig. 6.

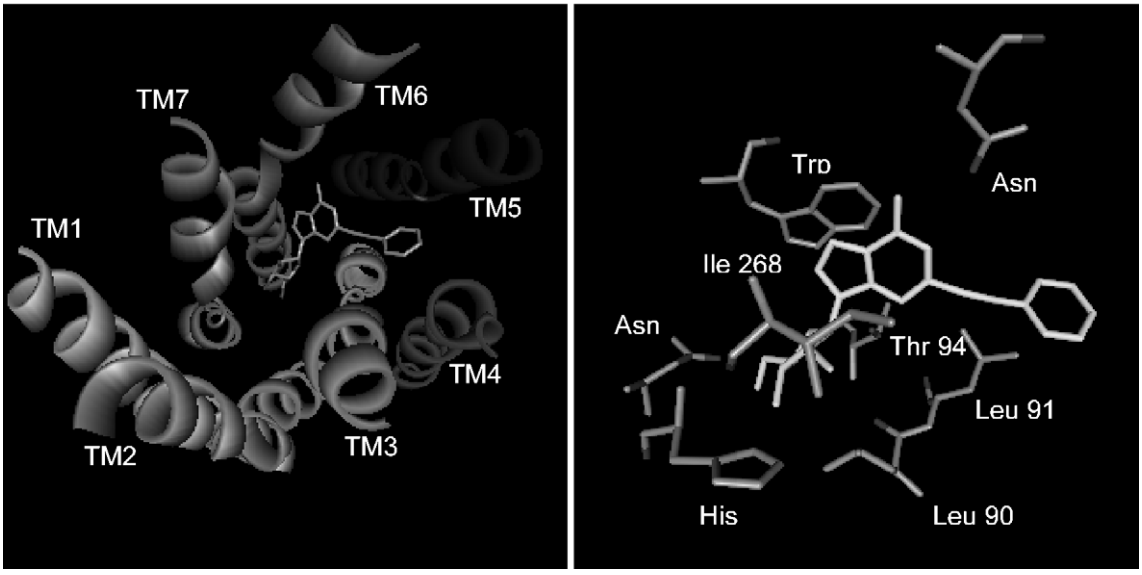


Fig. 6. The agonist 2-PEAdo docked into the seven *trans*-membrane domain of the human A_3 receptor. View from outside the cell (left) and details of the active site (right).

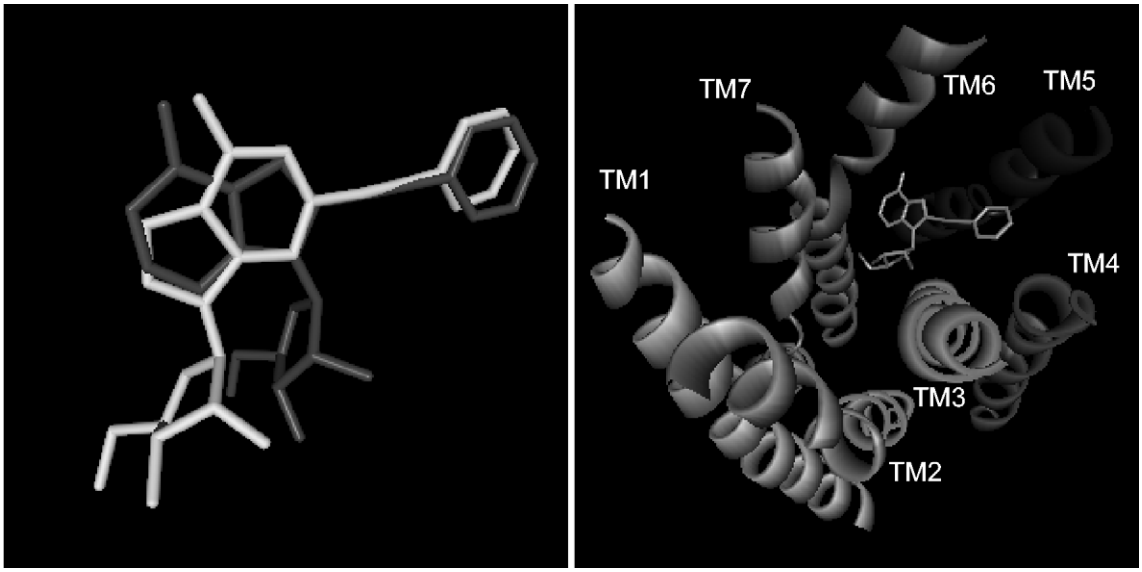


Fig. 7. Superimposition of 2- and 8-PEAdo (left) with the C2 of a molecule matching the C8 of the other one and *vice versa*, and relative docking of 8-PEAdo into the seven *trans*-membrane domain of the human A_3 receptor (right).

The alkynyl chain is accommodated in a hydrophobic region between the helices 3, 4 and 5. The ribose moiety, which is fundamental for the agonist activity, is in strict contact with the helices 3 and 7. In particular the 5′-hydroxyl group is within hydrogen-bond distance respect to Asn274 (TM7), while the 3′-hydroxy, which has been largely demonstrated to be required for full agonistic activity [9,43], is closely linked to the crucial His272 (TM7). The adenine region is found between the helices 3 and 6, with Asn250 (TM6) hydrogen-bonded to the amine in 6 position of the purine ring and Thr94 very close to N3. The receptor–ligand complex results to be more stable with respect to the separated molecules of about 60 kcal/mol.

On the contrary, 8-phenylethynyladenosine (8-PEAdo, **42**) cannot bind to the A_3 receptor in the same way of compound **28** because the alkynyl chain in C-8 position would overlap with the helix 6. The binding mode of this molecule, then, should be different.

We propose that the purine moieties of 2-PEAdo (**28**) and 8-PEAdo (**42**) can be superimposed in a way that the C2 of a molecule matches the C8 of the other one as showed in Fig. 7. In this way there is a very good steric and electrostatic correspondence at the level of the purine moieties and the phenylethynyl chains, even though there is not overlapping of the ribose moieties. As a consequence, 8-PEAdo is not able to stimulate the receptor response anymore, as the biological data in Table 2 clearly revealed.

These are probably the molecular bases that make the 8-alkynyladenosines the first example of adenosine receptor antagonist with nucleosidic structure and with an intact ribose moiety [35].

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