2- and 8-alkynyl-9-ethyladenines: Synthesis and biological activity at human and rat adenosine receptors

Rosaria Volpini1, Stefano Costanzi1, Catia Lambertucci1, Sauro Vittori1, Claudia Martini2, M. Letizia Trincavelli3, Karl-Norbert Klotz3 & Gloria Cristalli1

1 Dipartimento di Scienze Chimiche, Università di Camerino, Camerino, Italy; 2 Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Università di Pisa, Pisa, Italy; 3 Institut für Pharmakologie und Toxikologie, Universität Würzburg, Würzburg, Germany

Received 2 September 2004; accepted in revised form 21 October 2004

Key words: adenosine, adenosine antagonists, adenosine receptors, antagonist, purine derivatives, substituted adenines

Abstract

The synthesis of a series of 9-ethyladenine derivatives bearing alkynyl chains in 2- or 8-position was undertaken, based on the observation that replacement of the sugar moiety in adenosine derivatives with alkyl groups led to adenosine receptor antagonists. All the synthesized compounds were tested for their affinity at human and rat A1, A2A, and A3 adenosine receptors in binding assays; the activity at the human A2B receptor was determined in adenylyl cyclase experiments. Biological data showed that the 2-alkynyl derivatives possess good affinity and are slightly selective for the human A2A receptor. The same compounds tested on the rat A1 and A2A subtypes showed in general lower affinity for both receptors. On the other hand, the affinity of the 8-alkynyl derivatives at the human A1, A2A, and A2B receptors proved to be lower than that of the corresponding 2-alkynyl derivatives. On the contrary, the affinity of the same compounds for the human A3 receptor was improved, resulting in A3 selectivity. As in the case of the 2-alkynyl-substituted compounds, the 8-alkynyl derivatives showed decreased affinity for rat receptors. However, it is worthwhile to note that the 8-phenylethynyl-9-ethyladenine was the most active compound of the two series (Ki in the nanomolar range) at both the human and rat A3 subtype. Docking experiments of the 2- and 8-phenylethynyl-9-ethyladenines, at a rhodopsin-based homology model, gave a rational explanation of the preference of the human A3 receptor for the 8-substituted compound.

Introduction

Adenosine is an autacoid involved in the regulation of many aspects of cellular metabolism [1] and mediates its effects through the activation of at least four human receptors (P1), belonging to the superfamily of G protein-coupled receptors, which have been recently cloned [2] and classified as A1, A2A, A2B, and A3 [3]. All subtypes have been cloned from a variety of species including the rat and human. Species differences for the A3 adenosine receptor (AdoR) are larger than for other AR subtypes, particularly between rodent and human receptors (only 74% sequence identity between rat and human A3 amino acid sequence) [4]. This results in different affinities of ligands – particularly antagonists – for rat versus human A3 receptors [5, 6]. During the past decades many efforts have been directed toward the discovery of potent and selective adenosine receptor ligands aimed at finding novel drugs [7].

Most adenosine agonists possess a structure very close to that of the natural ligand adenosine; in contrast a wide range of structural classes have been characterized as adenosine receptor antagonists and developed as potential therapeutic agents for CNS disorders, inflammatory diseases, asthma, kidney failure and ischaemic injuries [8]. However, only a few xanthine antagonists as caffeine and theophylline have been approved as drugs for their CNS stimulating, diuretic, and bronchodilating effects, respectively [8, 9].

In many papers it has been demonstrated that introduction of alkynyl chains in the 2-position of adenosine derivatives led to compounds endowed with high affinity at all adenosine receptors (AdoRs) [10–15], whereas the introduction of the same alkynyl chains in the 8-position resulted in 8-alkynyladenosines, which were unable to stimulate [35S]GTPγS binding, and inhibited that stimulated by NECA, yielded A3 antagonists [16, 17].

Moreover, we have published the synthesis and activities for the human AdoR subtypes of a series of 9-ethyladenines, substituted at the 2-, 6- and 8-positions, which behaved as AdoR antagonists [18–20]. In fact, replacement of the sugar moiety of adenosine with a methyl group led to a rather unspecific antagonist of AdoR and adenosine itself is a weak adenosine antagonist [21].

Recently, substituted adenine derivatives, prepared as hypoglycemic agents, were found to possess high potency at the A2B AdoR subtype [22], whereas novel substituted
9-ethyladenine (11), isopropylamide (LDA), was used as the starting material. Analogue, while the reaction was successful when the 8-phenylhydroxypropynyl position were synthesized and tested at human and rat AdoR subtypes.

Materials and methods

Chemistry

Synthesis of the 2- and 8-alkynyl-9-ethylpurines 2-10, 12-19, and 22

The synthesis of the 2-alkynyl-9-ethyladenines 2-10 and 8-alkynyl-9-ethyladenines 12-19, was carried out starting from 2-iodo-9-ethyladenine (1; [18]) or 8-bromo-9-ethyladenine (11; [18]), respectively, and are reported in Schemes 1 and 2. In order to obtain the final products 1 or 11 was reacted with the suitable terminal alkyne using a modification of the palladium catalyzed cross-coupling reaction [18].

However, for the synthesis of the 8-alkynyl derivative 22, 8-iodo-9-ethyladenine (21), obtained through the iodination of the 9-ethyladenine (20) [18] with iodine and lithiumdiisopropylamide (LDA), was used as the starting material. This choice was due to the fact that reaction of 8-bromo-9-ethyladenine (11) with (R,S)-phenylhydroxypropyne failed to give the corresponding 8-phenylhydroxypropynyl analogue, while the reaction was successful when the 8-iodo analogue was used as starting material.

The synthesis of the derivatives 3, 9, 13, and 19 has been already published [18].

General synthetic procedures

Melting points were determined with a Büchi apparatus and are uncorrected. 1H NMR spectra were obtained with Varian Gemini 200 MHz or a Varian VXR 300-MHz spectrometer.
with CHCl₃–CH₃OH (98:2), gave, after crystallization from MeOH, 6, as white crystals. Yield 90%, m.p. > 250 °C; ¹H NMR (DMSO-d₆) δ 1.39 (t, 3H, J = 7.2 Hz, CH₃); 1.62 (m, 4H, H-cyclohexenyl); 2.15 (m, 4H, H-cyclohexenyl); 4.15 (q, 2H, J = 7.5 Hz, NCH₂); 6.30 (m, 1H, CH = C); 7.35 (bs, 2H, NH₂); 8.21 (s, 1H, H-8). Anal. Calcd. for C₁₅H₁₇N₅ (267.3): C, 67.39; H, 6.41; N, 26.20. Found: C, 67.68; H, 6.80; N, 26.01.

9-Ethyl-2-phenylethynyl-9H-purin-6-ylamine (7). The reaction of 1 with 1-phenylacetylene for 6 h, followed by chromatography on a silica gel column eluting with CH₂Cl₂–CH₃OH (99.5:0.5), gave, after crystallization from EtOH, 7, as white crystals. Yield 85%, m.p. 250–252 °C (dec); ¹H NMR (DMSO-d₆) δ 1.38 (t, 3H, J = 7.5 Hz, CH₃); 4.16 (q, 2H, J = 7.2 Hz, CH₂); 7.43 (m, 5H, H-Ph and NH₂); 7.58 (m, 2H, H-Ph); 8.23 (s, 1H, H-8). Anal. calcd. for C₁₅H₁₃N₅ (263.3): C, 68.42; H, 4.98; N, 26.60. Found: C, 68.78; H, 5.27; N, 26.35.

1-(4-(6-Amino-9-ethyl-9H-purin-2-ylethynyl)-phenyl)ethanone (8). The reaction of 1 with 1-(4-ethynylphenyl)ethanone for 6 h, followed by chromatography on a silica gel column eluting with CH₂Cl₂–CH₃OH (99:5:0.5), gave, after crystallization from MeOH, 8, as white crystals. Yield 85%, m.p. > 250 °C; ¹H NMR (DMSO-d₆) δ 1.04 (t, 3H, J = 7.2 Hz, CH₂CH₃); 1.35 (t, 3H, J = 7.2 Hz, NC₃H₂); 1.64 (m, 2H, CH₂CH₃); 2.57 (t, 2H, J = 6.9 Hz, C = CH₂); 4.20 (q, 2H, J = 7.2 Hz, CH₂OH); 7.37 (bs, 2H, NH₂); 8.39 (t, 1H, H-8). Anal. calcd. for C₁₇H₁₅N₅O (305.3): C, 60.21; H, 6.61; N, 27.04. Found: C, 60.43; H, 6.83; N, 26.76.

3-(6-Amino-9-ethyl-9H-purin-2-yl)-1-phenyl-prop-2-yn-1-ol (10). The reaction of 1 with (R,S)-1-phenyl-2-propyn-1-ol for 20 h, followed by chromatography on a silica gel column eluting with CHCl₃–CH₃OH (96:4), gave, after crystallization from EtOH, 10, as white crystals. Yield 57%, m.p. 225–227 °C; ¹H NMR (DMSO-d₆) δ 1.36 (t, 3H, J = 6.9 Hz, CH₂CH₃); 4.13 (q, 2H, J = 7.2 Hz, CH₂); 5.58 (d, 1H, J = 6.0 Hz, CH₂OH); 6.24 (d, 1H, J = 6.0 Hz, CHOH); 7.61 (m, 5H, H-Ph and NH₂); 8.19 (s, 1H, H-8). Anal. calcd. for C₁₇H₁₅N₅O (305.3): C, 66.87; H, 4.95; N, 22.94. Found: C, 66.98; H, 5.24; N, 22.78.

6-(6-Amino-9-ethyl-9H-purin-8-yl)-hex-5-yn-1-ol (11). The reaction of 11 with 5-hexynyl-1-ol for 48 h, followed by chromatography on a silica gel column eluting with CHCl₃–CH₃OH (95:5), gave, after crystallization from MeOH, 11, as white crystals. Yield 73%, m.p. 162–164 °C; ¹H NMR (DMSO-d₆) δ 1.35 (t, 3H, J = 7.2 Hz, CH₃); 1.63 (m, 4H, CH₂CH₃); 2.60 (t, 2H, J = 6.6 Hz, C = CÈH₂); 3.46 (m, 2H, CH₂CHOH); 4.20 (q, 2H, J = 7.5 Hz, NCH₂); 4.48 (t, 1H, J = 5.1 Hz, OH); 7.36 (bs, 2H, NH₂); 8.16 (s, 1H, H-2). Anal. calcd. for C₁₇H₁₇N₅O (293.3): C, 65.52; H, 5.15; N, 23.89. Found: C, 65.90; H, 5.29; N, 23.61.

Scheme 2
8-Cyclohexenylethynyl-9-ethyl-9H-purin-6-ylamine (15). The reaction of 11 with cyclohexylacetylene for 24 h, followed by chromatography on a silica gel column eluting with CHCl3–C6H12–CH3OH (55:40:5), gave after crystallization from MeOH, 15, as white crystals. Yield 85%, m.p. 247 °C (dec); 1H NMR (DMSO-d6) δ 1.26–1.83 (m, 13H, H-cyclohexyl and CH3); 2.79 (m, 1H, CH=C=C); 4.15 (q, 2H, J = 7.1 Hz, N–CH3); 7.33 (bs, 2H, NH2); 8.11 (s, 1H, H-2). Anal. Calcd. for C15H15N5O (293.3): C, 65.81; H, 5.27; N, 23.50.

8-Cyclohex-1-enylethynyl-9-ethyl-9H-purin-6-ylamine (16). The reaction of 11 with 1-ethynylcyclohexene for 36 h, followed by chromatography on a silica gel column eluting with CHCl3–CH3OH (99:1), gave, after crystallization from MeOH, 16, as white crystals. Yield 65%, m.p. 160 °C (dec); 1H NMR (DMSO-d6) δ 1.36 (t, 3H, J = 7.2 Hz, CH3); 1.64 (m, 4H, H-cyclohexyl); 2.21 (m, 4H, H-cyclohexenyl); 4.21 (q, 2H, J = 7.2 Hz, NCH2); 6.47 (m, 1H, CH=CH); 7.43 (bs, 2H, NH2); 8.18 (s, 1H, H-2). Anal. Calcd. for C17H17N5O (275.3): C, 66.89; H, 7.11; N, 25.79.

9-Ethyl-8-phenylethynyl-9H-purin-6-ylamine (17). The reaction of 11 with 1-phenylacetylene for 72 h, followed by chromatography on a silica gel column eluting with CH2Cl2–C6H12–CH3OH (85:10:5), gave, after crystallization from MeOH, 17, as white crystals. Yield 52%, m.p. 249–251 °C; 1H NMR (DMSO-d6) δ 1.43 (t, 3H, J = 7.2 Hz, CH3); 4.33 (q, 2H, J = 7.3 Hz, NCH2); 7.53 (m, 5H, H-Ph and NH2); 7.72 (m, 2H, H-Ph); 8.21 (s, 1H, H-2). Anal. Calcd. for C21H19N5O (363.3): C, 74.82; H, 4.98; N, 26.60. Found: C, 74.74; H, 5.65; N, 26.03.

1-[4-(6-Amino-9-ethyl-9H-purin-8-ylethynyl)-phenyl]-ethanone (18). The reaction of 11 with 1-(4-ethynylphenyl) ethanone for 16 h, followed by chromatography on a silica gel column eluting with CHCl3–CH3OH (90:10), gave, after crystallization from MeOH, 18, as white crystals. Yield 40%, m.p. > 250 °C; 1H NMR (DMSO-d6) δ 1.42 (t, 3H, J = 6.9 Hz, CH2CH3); 2.63 (s, 3H, COCH3); 4.34 (q, 2H, J = 6.9 Hz, CH2); 7.52 (bs, 2H, H-Ph); 7.85 (d, 2H, J = 8.4 Hz, H-Ph); 8.06 (d, 2H, J = 8.4 Hz, H-Ph); 8.21 (s, 1H, H-2). Anal. Calcd. for C17H13N5O (295.3): C, 66.87; H, 4.95; N, 22.94. Found: C, 67.23; H, 5.25; N, 22.78.

3-(6-Amino-9-ethyl-9H-purin-8-yl)-1-phenyl-prop-2-yn-1-ol (21). The reaction of 21 with (R,S)-1-phenyl-2-propyn-1-ol for 36 h, followed by chromatography on a silica gel column eluting with CHCl3–CH3OH (96:4), gave, after crystallization from CH2CN–MeOH, 22, as white crystals. Yield 13%, m.p. 226–228 °C; 1H NMR (DMSO-d6) δ 1.33 (t, 3H, J = 7.2 Hz, CH3); 4.21 (q, 2H, J = 7.2 Hz, NCH2); 5.77 (d, 1H, J = 6.1 Hz, CHOH); 6.49 (d, 1H, J = 6.1 Hz, CHOH); 7.41 (m, 5H, H-Ph and NH2); 7.59 (m, 2H, H-Ph); 8.19 (s, 1H, H-2). Anal. Calcd. for C21H15N5O (329.3): C, 65.52; H, 5.15; N, 23.88. Found: C, 65.81; H, 5.27; N, 23.50.

8.5Hz, CH3); 4.33 (q, 2H, J = 7.2 Hz, CH3); 1.64 (m, 4H, H-cyclohexyl); 2.21 (m, 4H, H-cyclohexenyl); 4.21 (q, 2H, J = 7.2 Hz, NCH2); 6.47 (m, 1H, CH=CH); 7.43 (bs, 2H, NH2); 8.18 (s, 1H, H-2). Anal. Calcd. for C17H17N5O (275.3): C, 66.89; H, 7.11; N, 25.79.

9-Ethyl-8-iodo-9H-purin-6-ylamine (21). To a solution of 2.2 ml (15.7 mmol) of freshly distilled diisopropylamine and 6.0 ml of dry THF, in a three necked round bottom flask, under a flux of N2, were added dropwise 9.8 ml (15.7 mmol) of butyllithium (1.6 M in hexane). The mixture was kept under stirring at r.t. for 15 min. After that, the mixture was cooled at −70 °C and 500 mg (3.1 mmol) of 20, dissolved in 10 ml of dry THF, were added, and after 1 h a solution of iodine (4.9 mmol) in 10 ml of dry THF was added dropwise.

After 1 h more at −70 °C 4 drops of glacial acetic acid and 3 ml of methanol were added. The mixture was allowed to warm at r. t., the solvent was removed in vacuo and the residue was chromatographed on a silica gel column eluting with CHCl3–CH3OH (98:2) to give, after crystallization from MeOH, 21, as white crystals. Yield 56%, m.p. 247–249 °C; 1H NMR (DMSO-d6) δ 1.31 (t, 3H, J = 7.2 Hz, CH3); 4.12 (q, 2H, J = 7.2 Hz, CH2); 7.33 (br s, 2H, NH2); 8.08 (s, 1H, H-2). Anal. Calcd. for C14H11N5 (289.1): C, 66.79; H, 4.61; N, 24.60. Found: C, 66.74; H, 5.65; N, 26.03.

Biological evaluation

Binding studies and adenylyl cyclase activity at human adenosine receptors

The radioligand binding experiments were carried out exactly as described previously [24]. For A1 adenosine receptor binding 1 nM [3H]CCPA was used as a radioligand, whereas 30 and 10 nM [3H]NECA was used for A2A and A3 receptors, respectively. Non specific binding was determined in the presence of 1 mM theophylline (A1) or 100 mM R-PIA (A2A and A3). KI values were calculated from competition curves by nonlinear curve fitting with the program SCTFIT [25]. CHO cells stably transfected with human adenosine receptors were grown adherently and maintained in Dulbecco’s Modified Eagles Medium with nutrient mixture F12 (DMEM/F12) without nucleosides, containing 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), l-glutamine (2 mM) and Genetin (G-418, 0.2 mg/ml) at 37 °C in 5% CO2/95% air as described earlier [24].

For radioligand binding studies and measurement of adenylyl cyclase activity crude membrane fractions were prepared from fresh or frozen cells with two different protocols which were described recently [24].

Determination of adenylyl cyclase activity followed the procedure described by [24]. IC50 values for the inhibition of cyclase stimulated with 5 μM NECA were calculated with the Hill equation and converted to Ki values with the Cheng and Prusoff equation [24]. The Hill slopes were near unity suggesting a competitive interaction of the antagonists tested.

Binding studies at rat adenosine receptors

A1 and A2A receptor binding: Displacement of [3H]CHA (31 Ci/mmol) from A1 adenosine receptor in rat cortical membranes and of [3H]CGS 21680 (42.1 Ci/mmol) from rat striatal membranes were performed as described [26].
Binding studies

All the compounds were evaluated at the human recombinant adenosine receptors, stably transfected into Chinese hamster ovary (CHO) cells, utilizing radioligand binding studies (A1, A2A, A3) or adenyl cyclase activity assay (A2B). Receptor binding affinity was determined using [3H]CCPA (2-chloro-N6-cyclopentyladenosine) as the radioligand for A1 receptors, whereas [3H]NECA (5′-N-ethylcarboxamidoadenosine) was used for the A2A and A3 subtypes [24]. In the case of A2B receptors KI values were calculated from IC50 values determined by inhibition of NECA-stimulated adenyl cyclase activity.

The compounds were also tested for their affinity at rat cortex A1, rat striatum A2A, and rat testis A3 subtypes, using [3H]CHA (N6-cyclopentyladenosine), [3H]CGS 21680 [2- [(2-carboxyethyl)phenylethylamino]-N-ethylcarboxamidoadenosine], and [125I]ABMECA [N6-(4-aminoo-3-iodobenzyl)adenosine-5′-N-methyluronamide] as the radioligands, respectively.

KI values are in μM with 95% confidence intervals in parentheses. The results are shown in Table 1.

Biological data showed that the 2-alkynyl derivatives possess good affinity at all human adenosine receptors and are slightly selective for the A2A subtype.

Compounds 5 and 10 bearing a cyclohexylethylene and a phenylhydroxypropyne in 2-position, respectively, resulted in the most active of the series with KI in the low nanomolar range both at human A1 and A2A receptors (5; KI A1 = 0.080 μM, KI A2A = 0.037 μM, and 10; KI A1 = 0.098 μM, KI A2A = 0.035 μM).

At the A2B receptor the derivatives bearing linear chains in 2-position showed functional activity in the μM range (2; KI A2B = 17 μM, 3; KI A2B = 12 μM, and 4; KI A2B = 19 μM), while the presence of sterically hindered substituents in the same position was detrimental for the activity. However, the presence of a phenylhydroxypropynyl chain seems to facilitate the interaction with the A2B subtype, in fact the 2-phenylhydroxypropynyl-9-ethyladenine resulted the most active compound of the two series (10; KI A2B = 1.4 μM). This finding is in agreement with our previous results obtained with the corresponding adenosine derivative. In fact, the (R,S)-2-phenylhydroxypropynyl-N-ethylcarboxamidoadenosine [(R,S)-PHPNECA, EC50 A2B = 1.1 μM] resulted in one of the most potent agonist with nucleoside structure at human A2B receptors reported so far [14, 29].

The affinity of the 2-alkynyl derivatives for the A3 subtype was lower than that at A1 and A2A receptors.

The same compounds, tested on the rat A1 and A2A subtypes, showed in general lower affinity for the rat A2A receptor, while compounds 7–9, bearing an aromatic ring in the 2-position, showed slightly higher affinity at the rat A1 receptor, compared to the corresponding human receptor, hence resulting slightly A1 selective (7; KI A1 (r) = 0.43 μM vs KI A1 (h) = 0.77 μM, 8; KI A1 (r) = 1.9 μM vs KI A1 (h) = 8.3 μM, and 9; KI A1 (r) = 0.15 μM vs KI A1 (h) = 0.21 μM). As in the case of human receptors, compounds 5 and 10 showed the highest affinity both at rat A1 and A2A subtypes (5; KI A1 = 0.072 μM, KI A2A = 0.069 μM, and 10; KI A1 = 0.14 μM, KI A2A = 0.14 μM).

At the rat A3 receptor these compounds showed comparable affinity to that at human one.

As far as the 8-alkynyl derivatives, their affinity at the human A1, A2A, and A2B receptors proved to be lower than
| Cpd | $R$ | $K_i$ ($\mu$M) or % inhibition | $K_i$ ($A_1^b$) | $K_i$ ($A_{2A}^c$) | $K_i$ ($A_{2B}^d$) | $K_i$ ($A_3^e$) |
|-----|-----|-------------------------------|------------|------------|------------|------------|
| 2   | -(CH$_2$)$_2$-CH$_3$ | 1.2 (h) 1.0–1.5 | 0.76 (h) (0.43–1.3) | 17 (h) (11–27) | 2.1 (h) (1.2–3.9) | 8% at 10 µM (r) |
| 3   | -(CH$_2$)$_3$-CH$_3$ | 0.55 (h) (0.24–1.2) | 0.42 (h) (0.26–0.69) | 12 (h) (5.9–27) | 2.3 (h) (1.1–4.9) | 1.3 (r) |
| 4   | -(CH$_3$)$_2$-OH | 3.6 (h) (2.9–4.6) | 1.9 (h) (1.1–3.3) | 19 (h) (10–35) | 14 (h) (6.5–32) | 17% at 10 µM (r) |
| 5   | cyclohexyl | 0.080 (h) (0.056–0.11) | 0.037 (h) (0.026–0.053) | > 30 (h) | 3.6 (h) (2.8–4.8) | 1.0 (r) |
| 6   | cyclohexenyl | 0.18 (h) (0.14–0.21) | 0.35 (h) (0.27–0.45) | > 30 (h) | 0.39 (h) (0.25–0.61) | 3.5 (r) |
| 7   | C$_6$H$_5$ | 0.77 (h) (0.36–1.6) | 0.40 (h) (0.24–0.67) | > 30 (h) | 0.52 (h) (0.35–0.78) | 3.0 (r) |
| 8   | C$_6$H$_4$p-COCH$_3$ | 8.3 (h) (5.1–14) | 3.8 (h) (1.9–7.7) | > 30 (h) | > 100 (h) | 11% at 10 µM (r) |
| 9   | -(CH$_2$)$_2$-C$_6$H$_5$ | 0.21 (h) (0.12–0.39) | 0.15 (h) (0.091–0.23) | > 30 (h) | 4.1 (h) (2.9–5.6) | 4.8 (r) |
| 10  | (R,S)-CH(OH)C$_6$H$_4$ | 0.098 (h) (0.092–0.10) | 0.035 (h) (0.018–0.072) | 1.4 (h) (0.85–2.3) | 4.3 (h) (3.0–6.2) | 1.2 (r) |
| 11  | -(CH$_2$)$_2$-CH$_3$ | 0.064 (h) (0.025–0.17) | 0.37 (h) (0.27–0.50) | 2.7 (h) (2.5–2.9) | 0.59 (h) (0.22–1.6) | 1.5 (r) |
| 12  | -(CH$_3$)$_3$-CH$_3$ | 2.3 (h) (1.4–3.9) | 0.44 (h) (0.22–0.87) | 22 (h) (11–45) | 0.62 (h) (0.34–1.1) | 3.5 (c) |
| 13  | -(CH$_2$)$_3$-CH$_3$ | 4.6 (h) (3.6–5.7) | 0.82 (h) (0.72–0.94) | 13 (r) (8.8–18) | 6.6 (h) (5.3–12) | 23 (r) |
| 14  | -(CH$_3$)$_3$-OH | 6.5 (h) (5.5–7.5) | 1.6 (h) (0.84–3.0) | 21 (h) (19–24) | 6.6 (h) (3.5–12) | 23 (r) |
| 15  | cyclohexenyl | 0.60 (h) (0.48–0.69) | 0.36 (h) (0.11–0.43) | > 100 (h) | 2.2 (h) (1.5–2.7) | nd (r) |
| 16  | cyclohexenyl | 1.2 (h) (0.66–2.1) | 2.0 (h) (1.2–3.4) | > 100 (h) | 0.43 (h) (0.23–0.80) | 2.2 (r) |

*Table 1. Affinities of 2- and 8-alkynyl-9-ethyladenines in radioligand binding assays at human and rat $A_1$, $A_{2A}$, and $A_3$ adenosine receptors and effects on adenylate cyclase activity at human $A_{2B}$ adenosine receptor.*
that of the corresponding 2-alkynyl analogues, with the exception of compound 12 (12; $K_i A_1 = 0.064 \mu M, K_i A_{2A} = 0.37 \mu M, K_i A_{2B} = 2.7 \mu M$, and $K_i A_3 = 0.59 \mu M$ vs 2; $K_i A_1 = 1.2 \mu M, K_i A_{2A} = 0.76 \mu M, K_i A_{2B} = 17 \mu M$, and $K_i A_3 = 2.1 \mu M$).

However, it is worthwhile to note that the derivatives, which showed some activity at the $A_{2B}$ receptor, are those bearing linear chains in the 8-position, as in the case of the 2-substituted compounds (12; $K_i A_{2B} = 2.7 \mu M$, 13; $K_i A_{2B} = 22 \mu M$, and 14; $K_i A_{2B} = 21 \mu M$).

On the contrary, the affinity of the 8-alkynyl derivatives for the human $A_3$ receptor, compared to the corresponding 2-substituted derivatives, was improved; hence the 8-phenylethynyl-9-ethyladenine (17) with a $K_i A_3$ (h) of 0.086 and 0.25 $\mu M$ (r) resulted the most active compound of the two series at both the human and rat $A_3$ subtype and was $A_3$ selective.

As in the case of the 2-substituted compound, the 8-alkynyl derivatives showed decreased affinity for rat receptors. The selectivity of this series of derivatives for the $A_1, A_{2A}$, and $A_3$ receptor subtypes was strictly correlated to the nature of the alkynyl chain, and there is a good correlation between affinity at human and rat receptors.

Molecular modeling

In order to get an insight into the mechanism of the ligand recognition and to give a rational explanation of the preference of the human $A_3$ receptor for the 8-substituted compounds rather than the 2-substituted compounds in this series, we carried out docking experiments of 2-phenylethynyl-9-ethyladenine (7) and 8-phenylethynyl-9-ethyladenine (17) at a rhodopsin-based homology model of the receptor (Figure 1).

In a previous molecular modeling study [17], we proposed that 2- and 8-alkynyl derivatives of adenosine bind to the human $A_3$ receptor in a way that the C2 of a molecule matches the C8 of the other one, with a good steric and electrostatic overlap of the purine moieties and the alkynyl chains.

On the basis of the structure activity relationships data, we hypothesized a similar binding mode for the alkynyl derivatives of adenosine and the correspondent 9-ethyladenine analogues. The results of our docking experiments were in good agreement with the biological data, confirming the soundness of the hypothesis on which they were based.

In the case of the adenosine derivatives, the compounds substituted at the 2-position were endowed with higher affinity than the correspondent 8-substituted analogues. In fact, in the latter case the sugar moiety had unfavourable negative ligand receptor interactions were eliminated even in the case of the 8-substituted compound. According to the results of the present study, in both situations the ethyl group is accommodated in the hydrophobic...
pocket formed by Leu 90 and Leu 91 of the third transmembrane domain (TM3). Furthermore, the binding of the 8-alkynyl derivative to the A3 receptor appears to be facilitated by a stronger electrostatic interaction of the crucial Asn 250 (TM6) with the adenine ring compared to the case of the 2-alkynyl derivatives (Figure 1).

Conclusions

With this study we have demonstrated that it is possible to modulate the activity at the adenosine receptor subtypes by introducing alkynyl chains in the 2- or 8-position of the 9-ethyladenine.

Although all the synthesized compounds were more active at human receptors, it is worthwhile to note that, in the series of the 2-substituted derivatives, compounds 5 and 10 are the ones endowed with the highest affinity at both human and rat A1 and A2A receptors.

The same trend has been observed with the 8-substituted derivatives: in fact the 8-phenylethynyl-9-ethyladenine (17) proved to be the most active and selective compound of the two series at human and rat A3 subtype.

From these results it is possible to conclude that the selectivity of these two series of derivatives for the A1, A2A, and A3 receptor subtypes is strictly correlated to the nature of the alkynyl chain, and that there is a good correlation between the affinity at human and rat receptors.

Acknowledgements

Supported by a grant from the Ministry of Research (COFIN, Grant no. 200061553, 2002) and by the University of Camerino (Fondo di Ricerca di Ateneo).

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