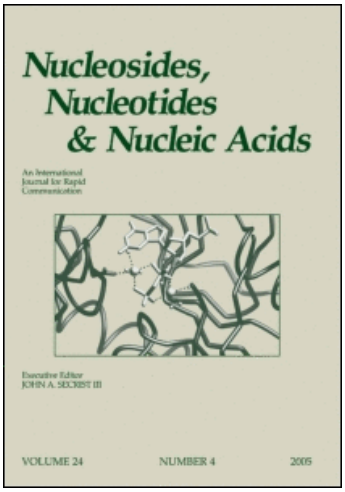

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SYNTHESIS AND ADENOSINE RECEPTOR AFFINITY AND POTENCY OF 8-ALKYNYL DERIVATIVES OF ADENOSINE

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ABSTRACT

Adenosine derivatives bearing different (ar)alkynyl chains at the 8-position were synthesized and tested at human adenosine receptors. Binding studies showed that all compounds possess affinity for the A_3 subtype in the high nM range. Moreover, guanosine 5′-O-(3-[^{35}S]thio)triphosphate binding assay indicated that the 8-alkynyl adenosines behaved as antagonists of NECA at A_3 receptors.

Adenosine (Ado) is a signalling molecule that mediates diverse biological effects via interaction with four different cell surface receptors termed A_1, A_{2A}, A_{2B} and A_3 (1). In recent years we demonstrated that C-2-substituted 5′-N-ethylcarboxamidoadenosine (NECA) are potent and selective ligands for adenosine receptor subtypes (2,3). 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 (4). In order to investigate the role of the carboxamido group at the 5′-position and to simplify the structure of these molecules, we synthesized adenosine derivatives bearing the above mentioned alkynyl chains at the C-2-position (5). Binding studies at human adenosine receptors showed that the 2-alkynyl derivatives of Ado possess good and comparable affinity at all receptor subtype in comparison to the corresponding NECA derivatives (5). These findings prompted us at investigating the effect of such substituent in a different position of adenosine; to this purpose we

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synthesized adenosine derivatives bearing in C-8-position the three above mentioned alkynyl chains. All the synthesized compounds were evaluated at human recombinant adenosine receptors, stably transfected into Chinese Hamster Ovary (CHO) cells, utilizing radioligand bindings (A_1, A_{2A}, A_3) or adenylyl cyclase activity assays (A_{2B}) (6). Preliminary results showed that in all cases the introduction of an alkynyl chain at the C-8 position of Ado is detrimental for the affinity and potency at A_1, A_{2A}, and A_{2B} receptors, while is more tolerated by the A_3 receptor (Ki A_3 in the high nM range).

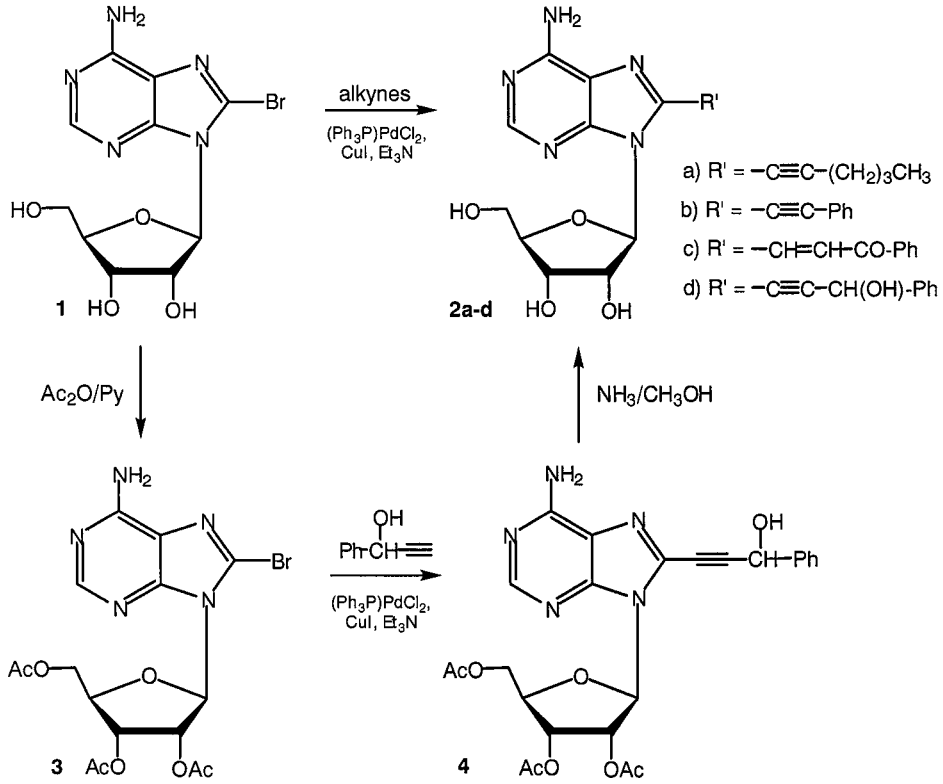
Furthermore, the intrinsic activity of the C-8-alkynylAdos were determined in guanosine 5′-O-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding assay in membranes of CHO cells which express the human A_3 receptor subtype. NECA (1 μM) was used as reference full agonist and its stimulation of [^{35}S]GTPγS binding to the G protein was set to 100% (7). Surprisingly, the C-8-alkynylAdos behaved as A_3 adenosine antagonists. In fact they did not stimulate basal GTPγS binding, but inhibited to various extent NECA-stimulated binding.

CHEMISTRY

The synthesis of 8-substituted adenosines (**2a**–**d**) was accomplished starting from commercial 8-bromoadenosine (8-BrAdo, **1**). Substitution of the bromine in the 8-position of **1** with the three different alkynyl chains (commercially available hexyne, phenylethyne or phenylhydroxypropyne) was carried out by a modification of the palladium catalyzed cross-coupling reaction (2) described in the general method (Scheme 1). Reaction of **1** with phenylhydroxypropyne did not give the corresponding 8-phenylhydroxypropyne (**2d**) but a product deriving from a tautomeric rearrangement on the side chain (**2c**). The structure of (*Z*)-8-(3-keto-3-phenyl-1-propen-1-yl)-9-(β-D-ribofuranosyl)adenine (**2c**) was confirmed by ^{1}H NMR and ^{13}C NMR. In fact the ^{1}H NMR displayed two doublets of the (Z) double bond protons at δ 7.87 and 8.22 with a coupling constant of 15.2 Hz, while the ^{13}C NMR showed a carbonyl group signal at 188.4 ppm. The same reaction, performed on the acetylated 8-bromoAdo (**3**) (8) as reported in Scheme 1, was successful and gave compound **4** which was deprotected with methanolic ammonia to obtain the desired 8-phenylhydroxy propynylAdo (**2d**), although in very low yield.

EXPERIMENTAL

Melting points were determined with a Büchi apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained with Varian VXR 300 MHz spectrometer; δ in ppm, J in Hz. All exchangeable protons were confirmed by addition of D_2O. TLC were carried out on pre-coated TLC plates with silica gel 60 F-254 (Merck). For column chromatography, silica gel 60 (Merck) was used. Elemental analyses



Scheme 1.

were determined on Carlo Erba model 1106 analyser and are within ±0.4% of theoretical values.

General method for the synthesis of 8-(ar)alkynyladenosines 2a–c, and **4**. To a solution of **1** or **3** (0.51 mmol) in dry DMF (15 mL), and Et_3N (2.3 mL) under an atmosphere of N_2 were added bis(triphenylphosphine)palladium dichloride (8.1 mg, 0.012 mmol) and CuI (0.51 mg, 0.003 mmol). The appropriate terminal alkyne (3.1 mmol) was added and the reaction mixture was stirred under an atmosphere of N_2 at room temperature for the time reported for each compound. The solvent was removed *in vacuo* and the residue was chromatographed on a silica gel column or TLC plates eluting with a suitable mixture of solvents to give the desired derivatives **2a**–**c**, or **4** as amorphous solids.

8-(1-Hexyn-1-yl)-9-(β-D-ribofuranosyl)adenine (2a). The reaction of **1** with 1-hexyne for 16 h, followed by chromatography on a silica gel column eluted with $CHCl_3$-MeOH (92:8), gave **2a** (138 mg; 69%); ^1H NMR (Me_2SO-d_6) δ 0.94 (t, $J = 7.1$ Hz, 3H, CH_3), 1.53 (m, 4H, $(CH_2)_2$-CH_3), 2.59 (t, $J = 6.7$ Hz, 2H,

CH_2-C≡C), 3.62 (m, 2H, CH_2-5′), 3.99 (m, 1H, H-4′), 4.22 (m, 1H, H-3′), 5.03 (m, 1H, H-2′), 5.95 (d, $J = 6.9$ Hz, 1H, H-1′), 7.50 (bs, 2H, NH_2), 8.15 (s, 1H, H-8). Anal. calcd. for $C_{16}H_{21}N_5O_4$ (347.37): C 55.32, H 6.09, N 20.16; found: C 55.25, H 6.00, N 20.28.

8-(Phenylethyn-1-yl)-9-(β-D-ribofuranosyl)adenine (2b). The reaction of **1** with phenylethyne for 48 h, followed by chromatography on a silica gel column eluted with $CHCl_3$-MeOH (92:8), gave **2b** (153 mg; 72%); ^{1}H NMR (Me_2SO-d_6) δ 3.64 (m, 2H, CH_2-5′), 4.03 (m, 1H, H-4′), 4.23 (m, 1H, H-3′), 5.04 (m, 1H, H-2′), 6.07 (d, $J = 6.7$ Hz, 1H, H-1′), 7.55 (m, 3H, H-Ph), 7.66 (m, 4H, H-Ph and NH_2), 8.20 (s, 1H, H-8). Anal. calcd. for $C_{18}H_{17}N_5O_4$ (367.36): C 58.85, H 4.66, N 19.06; found: C 55.50, H 4.56, N 19.16.

(*Z*)-8-(3-Keto-3-phenyl-1-propen-1-yl)-9-(β-D-ribofuranosyl)adenine (2c). The reaction of **1** with 3-hydroxy-3-phenyl-1-propyne for 4 days, followed by chromatography on a silica gel TLC plate eluted with $CHCl_3$-MeOH (85:15), gave **2c** (92 mg; 45%); ^{1}H NMR (Me_2SO-d_6) δ 3.64 (m, 2H, CH_2-5′), 4.04 (m, 1H, H-4′), 4.20 (m, 1H, H-3′), 4.89 (m, 1H, H-2′), 6.10 (d, $J = 6.6$ Hz, 1H, H-1′), 7.69 (m, 5H, H-Ph and NH_2), 7.87 (d, $J = 15.2$ Hz, 1H, CH), 8.08 (d, $J = 7.0$, 2-H, H-Ph), 8.18 (s, 1H, H-2). 8.22 (d, $J = 15.2$ Hz, 1H, CH), ^{13}C NMR (Me_2SO-d_6) δ 61.9 (C-5′), 70.6 (C-3′), 72.3 (C-2′), 86.6 (C-4′), 87.8 (C-1′), 119.5 (C-5), 128.0 (*C* = C), 128.2 (C-Ph), 128.4 (C-Ph), 128.6 (C-Ph), 129.1 (C-Ph), 133.7 (*C* = C), 136.9 (C-Ph), 145.6 (C-8), 150.0 (C-4), 153.1 (C-2), 156.2 (C-6), 188.4 (CO). Anal. calcd. for $C_{19}H_{19}N_5O_5$ (397.38): C 57.43, H 4.82, N 17.62; found: C 57.38, H 4.77, N 17.82.

8-(3-Hydroxy-3-phenyl-1-propyn-1-yl)-9-(2,3,5-tri-O-acetyll-β-D-ribofuranosyl) adenine (4). The reaction of **3** (8) with 3-hydroxy-3-phenyl-1-propyne for 3 days, followed by chromatography on a silica gel column eluted with $CHCl_3$-MeOH (96:4), gave **4** (34 mg; 13%); ^{1}H NMR (Me_2SO-d_6) δ 1.96 (s, 3H, $COCH_3$), 2.04 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$), 4.17 (m, 1H, CH_2-5′), 4.40 (m, 2H, CH_2-5′ and H-4′), 5.75 (m, 2H, H-1′ and H-3′), 6.17 (m, 2H, H-2′ and *CH*-OH), 7.43 (m, 3H, H-Ph), 7.59 (m, 4H, H-Ph and NH_2), 8.22 (s, 1H, H-2). Anal. calcd. for $C_{25}H_{25}N_5O_8$ (523.49): C 57.36, H 4.81, N 13.38; found: C 57.33, H 4.75, N 13.52.

8-(3-Hydroxy-3-phenyl-1-propyn-1-yl)-9-(β-D-ribofuranosyl)adenine (2d). A mixture of **4** (0.07 mmol) and methanolic ammonia (3 mL) was stirred at room temperature for 30 min. The reaction mixture was evaporated and the residue was chromatographed on a silica gel TLC plate eluted with $CHCl_3$-MeOH (80:20) to give **2d** (6 mg; 23%); ^{1}H NMR (Me_2SO-d_6) δ 3.61 (m, 2H, CH_2-5′), 3.98 (m, 1H, H-4′), 4.21 (m, 1H, H-3′), 5.03 (m, 1H, H-2′), 5.77 (d, $J = 5.7$ Hz, 1H, *CH*-OH), 5.99 (d, $J = 6.7$ Hz, 1H, H-1′), 7.44 (m, 2H, H-Ph), 7.62 (m, 5H,

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H-Ph and NH_2), 8.17 (s, 1H, H-2). Anal. calcd. for $C_{19}H_{19}N_5O_5$ (397.38): C 57.43, H 4.82, N 17.62; found: C 57.23, H 4.79, N 17.82.

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