

THE SYNTHESIS OF NEW ADENOSINE A₃ SELECTIVE LIGANDS CONTAINING BIOISOSTERIC ISOXAZOLES

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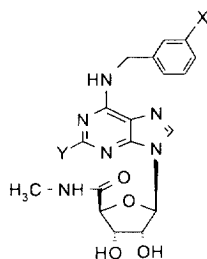
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Abstract. The synthesis and purinergic receptor binding of novel adenosine A₃ ligands is described. Many selective A₃ receptor agonists e.g. *N*-(3-iodobenzyl)adenosine-5-methyluronamide (IB-MECA) contain a 4'-ribosylalkylamide moiety. We found that this amide and other 4'-functional groups could be replaced with an isosteric isoxazole, and the target molecules retained potent binding to the recombinant human A₃ receptor.
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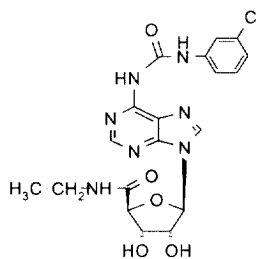
Adenosine is a naturally occurring purine nucleoside that has a variety of well-documented regulatory functions and physiological effects. The pharmacologically distinct adenosine (P₁) receptor subtypes are known as A₁, A_{2A}, A_{2B} (of high and low affinity), and A₃.¹ Since the discovery of the A₃ receptor early this decade^{2,3} an effort has been made to identify ligands at this subtype,⁴ but very few selective A₃ agonists have been described to date.



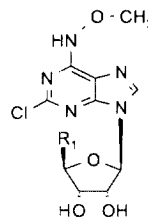
1, X = Y = H, *N*-benzyl MECA

2, X = I, Y = H, IB-MECA

3, X = I, Y = Cl, 2-Cl IB-MECA



4



5, R₁ = Cl-CH₂-, NNC 21-0113

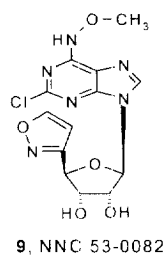
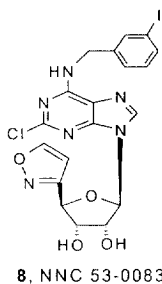
6, R₁ = CH₂=CH-, NNC 21-0238

7, R₁ = CH₃OCH₂-, NNC 53-0055

The first A₃ agonists to be discovered,^{5,6} represented by structures 1–3, contain a bulky purine 6-amino substituent (a benzyl group) along with a 4'-alkylamide moiety. Potency is increased further by a 3-iodine, as in 2 or 3. This theme of bulky amino substituents was also reflected in a later series⁷, in which compound 4 was the most potent. The recently revealed⁸ A₃ ligands 5–7 represent a structural departure compared to 1–4, since they contain both the smaller *N*-methoxy substituent and a new range of ribose 5'-modifications. Potential future therapeutic applications of adenosine A₃ ligands appear to be involvement with the control of cytokines, [such as tumor necrosis factor alpha (TNF-α)⁹], as anti-inflammatory agents or in the treatment of asthma.⁴

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The use of five-membered heterocycles as isosteric replacements for esters and amides is well established.^{10–12} As part of our strategy of incorporating new functionalities in the design of novel adenosine receptor agonists^{13,14} we decided to investigate replacement of the methylamide in the selective A₃ agonist 2-chloro-*N*-(3-iodobenzyl)adenosine-5-methyluronamide (2-Cl-IB-MECA) **3** with an isoxazole moiety. Compound **8** is thereby analogous to compound **3**, and the *N*-alkoxypurine **9** has structural similarities to compounds **5–7**.

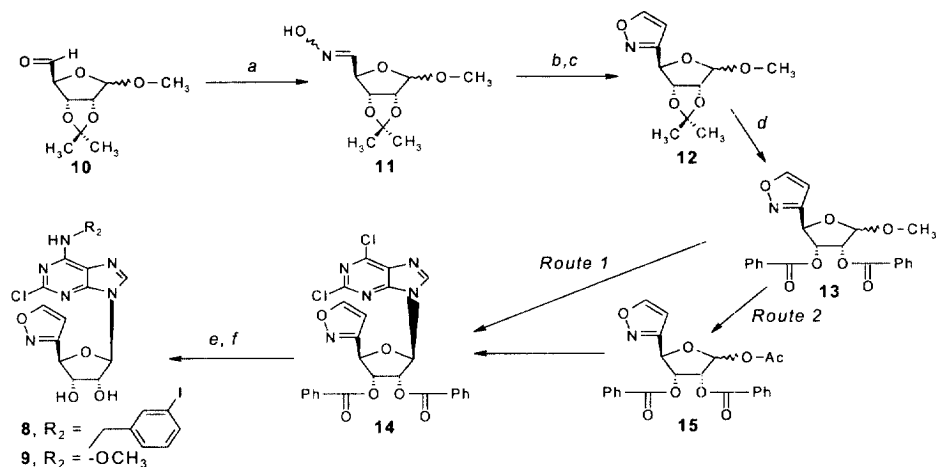


Chemistry

The synthesis of the novel isoxazole derivatives **8** and **9** is shown in Scheme 1. We opted for a strategy in which the isoxazole was first assembled at the ribose 4-position, and the other heterocycle, a 9*H*-purine, was introduced subsequently. The oxime **11**, derived from the ribose aldehyde **10**,¹⁵ was isolated as an *E/Z* mixture, and from this the isoxazole **12** was built in two steps.¹⁶ First the corresponding nitrile oxide was generated from **11**, and a 1,3-dipolar addition was performed using either TMS-acetylene or vinyl acetate as dipolarophile. The addition products were treated with aqueous sodium hydroxide or DBU to provide the isoxazole **12**. Once the heterocycle was in place, the 1-methylethylidene group was hydrolyzed under acidic conditions and the 2- and 3-hydroxyls were reprotected as the dibenzoate **13**.¹⁷ Attachment of the 2,6-dichloro-9*H*-purine to the ribose moiety was achieved by two different methods. The first method (Route 1, Scheme 1), utilized the 1-*O*-methyl sugar **6** and Vorbruggen's procedure^{18,19} with silylated dichloropurine gave 2,6-dichloro-9-[2,3-di-*O*-benzoyl-4-(3-isoxazolyl)-β-*D*-erythrofuranosyl]-9*H*-purine **14** in low yield. The more successful method (Route 2, Scheme 1) involved replacing the ribose 1-*O*-methyl with a 1-*O*-acetyl group, and reacting **15** with 2,6-dichloro-9*H*-purine in an acid-catalyzed process¹³ at 140 °C *in vacuo*, provided the key intermediate **14** in good yield. The 6-chloro atom in **14** was displaced with either 3-iodobenzylamine or *O*-methylhydroxylamine, which after de-blocking provided the target purine derivatives **8** and **9** containing the isosteric ribose 4-isoxazole.

Pharmacological Data

The novel purine derivatives **8** and **9** were evaluated *in vitro* in adenosine A₁ and A_{2A} binding assays utilizing published procedures.^{6,19} For A₃ receptor binding, the human A₃ receptor was expressed in a human embryonic kidney cell line (HEK 293) and [¹²⁵I]-AB-MECA binding was performed as described below (see reference 19); data for **3** in Table 1 is from ref. 4. The data displayed in Table 1 shows that the isoxazoles **8** and **9** display selectivity in binding towards human A₃ receptors, with relatively weak binding observed to A₁ and A_{2A} receptors.

Scheme 1. Synthesis of adenosine A₃ receptor ligands 8 and 9.

Reagents and conditions: (a) NH₂OH.HCl, pyridine; (b) NBS, (CH₃)₃Si-acetylene, Et₃N, 0 °C, 24 h (44%) or NBS, vinyl acetate, Et₃N, 0 °C, 24 h; (c) 1N NaOH, used in the (CH₃)₃Si case (57%) or DBU, THF used in the vinyl acetate case (65% for 2 steps); (d) Dowex 50 H⁺, benzoyl chloride, pyridine, DMAP, 0 °C, 12 h (56% for 2 steps). Route 1: 2,6-dichloro-9H-purine, 1,1,1,3,3,3-hexamethyldisilazane, (CH₃)₃Si-triflate, 12 h (12%); Route 2: (1) Ac₂O, AcOH, H₂SO₄, 10 h; (2) 2,6-dichloro-9H-purine, 140 °C, vacuum, 5 h (66%); (e) CH₃ONH₂.HCl, Et₃N, 1,4-dioxan, reflux, 20 h or 3-I-PhCH₂NH₂.HCl, Et₃N, 1,4-dioxan, reflux; (f) 10% NH₃ in CH₃OH, 4 h (55% in the case of **8** and 34% in the case of **9**).

Table 1. Adenosine receptor binding

Structure	A ₁ receptor, K _i (nM) (Inhibition of rat brain [³ H]-R-PIA binding)	A _{2A} receptor, K _i (nM) (Inhibition of rat striatum [³ H]-CGS 21680 binding)	A ₃ receptor, K _i (nM) (Inhibition of [¹²⁵ I]-AB-MECA binding, human receptor)
1	2400	2100	41
3	54	56	1.1
5	74	4650	26
6	1230	>10000	20
8	1900	1900	7.8
9	620	>10000	31

We conclude from the above data that the isoxazole moiety in **8** is able to act as a bioisostere for methylamides such as **1** and **3**¹ and that the same heterocycle is also able to replace the 5'-substituents in A₃ ligands **5** - **7**. Functional studies are in progress to establish whether **8** and **9** act as adenosine A₃ agonists or antagonists.

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17. Physical data for selected compounds: 2,3-Dibenzoyl-1-*O*-methyl- β -D-erythrofuranos-4-yl)-isoxazole **13**, *m/z* 409.11570 (M⁺), C₂₂H₁₉NO₇ requires 409.11615; δ_{H} (400 MHz, CDCl₃) 3.52 (3H, s, CH₃O), 5.22 (1H, d, *J* = 1 Hz, H-1), 5.59 (1H, dd, *J* = 1 and 7.5 Hz, H-2), 5.74 (1H, d, *J* = 5 Hz, H-4), 5.95 (1H, dd, *J* = 5 and 7.5 Hz, H-3), 6.50 (1H, d, *J* = 2 Hz, H-4 in isoxazole), 7.50 (6H, m, Ph H-3, H-4 and H-5), 7.90 (2H, dd, *J* = 1 and 7 Hz, Ph H-2 and H-6), 8.02 (2H, dd, *J* = 1 and 7 Hz, Ph H-2 and H-6), 8.45 (1H, d, *J* = 2 Hz, isoxazole H-4); δ_{C} (100 MHz, CDCl₃) 55.29, 74.57, 74.77, 74.98, 102.33, 106.45, 127.88, 127.98, 128.05, 128.28, 128.33, 128.59, 129.32, 129.35, 129.66, 132.98, 133.11, 158.75, 161.79, 164.74. 2,6-Dichloro-9-[2,3-di-*O*-benzoyl-4-(3-isoxazolyl)- β -D-erythrofuranosyl]-9*H*-purine **14**, *m/z* 565.05813 (M⁺), C₂₆H₁₇N₅O₆Cl₂ requires 565.05559; δ_{H} (400 MHz, CDCl₃) 5.71 (1H, d, *J* = 3.5 Hz, H-1'), 6.25 (2H, m, H-2' and H-3'), 6.21 (1H, d, *J* = 5 Hz, H-4'), 6.70 (1H, d, *J* = 1.5 Hz, isoxazole H-4), 7.5 (6H, m, H-3, 2 x Ph H-4 and H-5), 7.90 (2H, dd, *J* = 1 and 7.5 Hz, Ph H-2 and H-6), 8.10 (2H, dd, *J* = 1 and 7.5 Hz, Ph H-2 and H-6), 8.52 (1H, d, *J* = 1.5 Hz, isoxazole H-4), 8.60 (1H, s, H-8); δ_{C} (100 MHz, CDCl₃) 73.80, 74.01, 77.23, 87.12, 102.76, 128.01, 128.10, 128.13, 128.21, 129.39, 129.44, 129.80, 130.01, 133.52, 133.58, 144.31, 151.87, 152.33, 152.36, 152.80, 159.45, 159.54, 164.67, 164.77. 2-Chloro-9-[4-(3-isoxazolyl)- β -D-erythrofuranosyl]-*N*-methoxyadenine **9**, *m/z* 368.06289 (M⁺), C₁₅H₁₃N₆O₅Cl requires 368.06360. δ_{H} (400 MHz, DMSO-*d*₆) 3.72 (3H, s, CH₃O), 4.51 (1H, m, H-3'), 4.72 (1H, m, H-2'), 5.05 (1H, dd, *J* = 5, H-4'), 5.74 (1H, d, *J* = 6, 3'-OH), 5.78 (1H, d, *J* = 6, 2'-OH), 6.02 (1H, d, *J* = 5, H-1'), 6.81 (1H, s, *J* = 2, isoxazole H-4), 8.41 (1H, s, N-H), 8.96 (1H, s, *J* = 2, isoxazole H-5); 8.50 (1H, s, H-8); δ_{C} (100 MHz, DMSO-*d*₆) 56.42, 74.71, 76.11, 76.90, 84.75, 101.89, 146.01, 150.33, 151.83, 152.78, 153.65, 160.34, 161.24. 2-Chloro-*N*-3-iodobenzyl-9-[4-(3-isoxazolyl)- β -D-erythrofuranosyl]adenine **8**, *m/z* 553.99893 (M⁺), C₁₉H₁₆N₆O₄ICl requires 553.99663; δ_{H} (400 MHz, CDCl₃) 4.85 (5H, m, *N*-CH₂, H-2', H-3' and 3'-OH), 5.43 (1H, d, *J* = 4 Hz, H-4'), 6.05 (1H, d, *J* = 5.5 Hz, H-1'), 6.49 (1H, d, *J* = 2 Hz, isoxazole H-4), 7.09 (1H, dd, *J* = 8 Hz, Ph H-5), 7.35 (1H, d, *J* = 8 Hz, Ph H-6), 7.63 (1H, d, *J* = 8 Hz, Ph H-4), 7.72 (1H, s, Ph H-2), 7.91 (1H, s, H-8), 8.50 (1H, d, *J* = 2 Hz, isoxazole H-5). For full experimental details, see Knutsen, L. J. S.; Olsen, U. B.; Roberts, S. M.; Varley, D. R.; Bowler, A. N. WO 98/01459A1 (15.01.1998).
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