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THE SYNTHESIS OF NEW ADENOSINE A_3 SELECTIVE LIGANDS CONTAINING BIOISOSTERIC ISOXAZOLES

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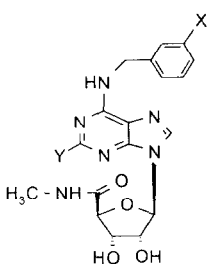
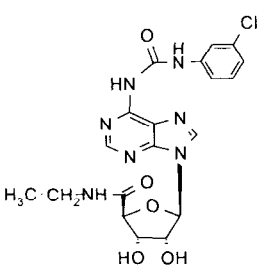
Abstract. The synthesis and purinergic receptor binding of novel adenosine A_3 ligands is described. Many selective A_3 receptor agonists e.g. *N*-(3-iodobenzyl)adenosine-5'-methyluronamide (IB-MECA) contain a 4'-ribosylalkylamide moiety. We found that this amide and other 4'-functional groups could be replaced with an isosteric isoxazole, and the target molecules retained potent binding to the recombinant human A_3 receptor.

Adenosine is a naturally occurring purine nucleoside that has a variety of well-documented regulatory functions and physiological effects. The pharmacologically distinct adenosine (P_1) receptor subtypes are known as A_1, A_{2A}, A_{2B} (of high and low affinity), and A_3.[1] Since the discovery of the A_3 receptor early this decade[2,3] an effort has been made to identify ligands at this subtype,[4] but very few selective A_3 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



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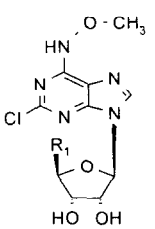

5, R_1 = Cl-CH_2-, NNC 21-0113
6, R_1 = CH_2=CH-, NNC 21-0238
7, R_1 = CH_3OCH_2-, NNC 53-0055

The first A_3 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 series[7], in which compound **4** was the most potent. The recently revealed[8] A_3 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 A_3 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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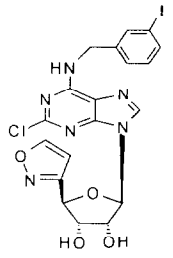
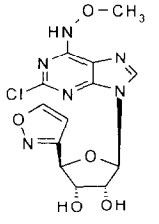
The use of five-membered heterocycles as isosteric replacements for esters and amides is well established.[10-12] As part of our strategy of incorporating new functionalities in the design of novel adenosine receptor agonists[13,14] we decided to investigate replacement of the methylamide in the selective A_3 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**.



8, NNC 53-0083



9, NNC 53-0082

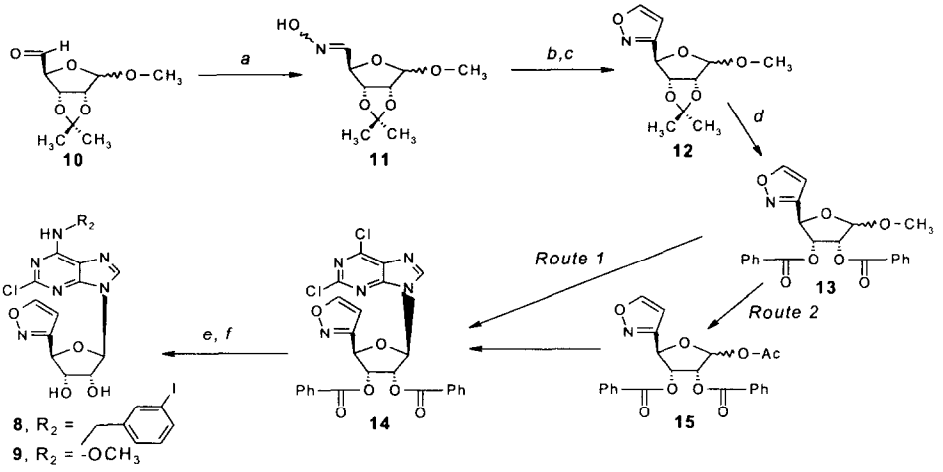
Chemistry

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-*erythro*furanosyl]-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 process[13] 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 A_1 and A_{2A} binding assays utilizing published procedures.[6,19] For A_3 receptor binding, the human A_3 receptor was expressed in a human embryonic kidney cell line (HEK 293) and [^{125}I]-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 A_3 receptors, with relatively weak binding observed to A_1 and A_{2A} receptors.

Scheme 1. Synthesis of adenosine A_3 receptor ligands 8 and 9.



Reagents and conditions: *(a)* $NH_2OH.HCl$, pyridine; *(b)* NBS, $(CH_3)_3Si$-acetylene, Et_3N, 0 °C, 24 h (44%) or NBS, vinyl acetate, Et_3N, 0 °C, 24 h; *(c)* 1N NaOH, used in the $(CH_3)_3Si$ 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-9*H*-purine, 1,1,1,3,3,3-hexamethyldisilazane, $(CH_3)_3Si$-triflate, 12 h (12%); Route 2: *(1)* Ac_2O, AcOH, H_2SO_4, 10 h; *(2)* 2,6-dichloro-9*H*-purine, 140 °C, vacuum, 5 h (66%); *(e)* $CH_3ONH_2.HCl$, Et_3N, 1,4-dioxan, reflux, 20 h or 3-I-$PhCH_2NH_2.HCl$, Et_3N, 1,4-dioxan, reflux; *(f)* 10% NH_3 in CH_3OH, 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**[4] and that the same heterocycle is also able to replace the 5'-substituents in A_3 ligands **5** - **7**. Functional studies are in progress to establish whether **8** and **9** act as adenosine A_3 agonists or antagonists.

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17. Physical data for selected compounds: 2,3-Dibenzoyl-1-*O*-methyl-β-D-*erythro*furanos-4-yl)-isoxazole **13**, *m/z* 409.11570 (M^+). $C_{22}H_{19}NO_7$ requires 409.11615; δ_H(400 MHz, $CDCl_3$) 3.52 (3H, s, CH_3O), 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, $CDCl_3$) 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-*erythro*furanosyl]-9*H*-purine **14**, *m/z* 565.05813 (M^+), $C_{26}H_{17}N_5O_6Cl_2$ requires 565.05559; δ_H(400 MHz, $CDCl_3$) 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 (100MHz, $CDCl_3$) 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-*erythro*furanosyl]-*N*-methoxyadenine **9**, *m/z* 368.06289 (M^+), $C_{13}H_{13}N_6O_5Cl$ requires 368.06360. δ_H(400 MHz, DMSO-d_6) 3.72 (3H, s, CH_3O), 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-*erythro*furanosyl]adenine **8**, *m/z* 553.99893 (M+), $C_{19}H_{16}N_6O_4ICl$ requires 553.99663; δ_H(400 MHz, $CDCl_3$) 4.85 (5H, m, *N*-CH_2, 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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