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THE DESIGN AND SYNTHESIS OF NOVEL ADENOSINE AGONISTS

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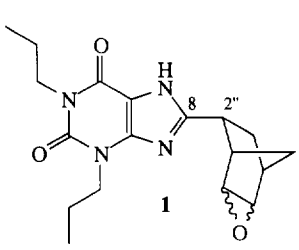
Abstract: The 2*R* and 2*S-endo* isomers of N^6-(5,6-epoxynorborn-2-yl)adenosine have been synthesised and shown to be potent agonists for the A_1 adenosine receptor. The 2*S-endo* isomer was equipotent to N^6-cyclopentyladenosine and 10- to 12-fold more potent than the 2*R-endo* isomer. Copyright © 1996 Elsevier Science Ltd

Adenosine, marketed as Adenocard™, is currently in use for the treatment of paroxysmal supraventricular tachycardia (PSVT).[1] Due to adenosine's rapid clearance from the blood up to 35% of tachycardias recur within two minutes after termination.[2] This limitation maybe overcome through the use of longer acting adenosine agonists with high selectivity for the A_1 adenosine receptor (A_1AdoR). A recent study on adenosine antagonists, which identified ENX (**1**) as the most A_1AdoR selective of the alkylxanthine adenosine antagonists,[3] has provided a lead for the development of such compounds. Structure/activity relationships and receptor models indicate that the C8 substituent of alkylxanthines and N^6 substituent of adenosines access the same area of the A_1AdoR.[4,5] Further, it is known that N^6-substituted adenosines are potent A_1 selective adenosine agonists with resistance to metabolic degradation.[1,6] As a result we have targeted compounds substituted in the N^6 position with a 5,6-epoxynorbornyl group as potentially highly A_1 selective, longer acting adenosine agonists. In an initial study, we reported the synthesis of the (2*R,S*)-*exo* and (2*R,S*)-*endo* isomers of N^6-(5,6-epoxynorborn-2-yl)adenosine (ENAdo, **2**).[7] Since the N^6-substituent binding has been well documented to have a marked stereochemical requirement,[1] we now describe the asymmetric synthesis and biological activity of the 2*R* and 2*S* isomers of the more potent N^6-(*endo*-5,6-epoxynorborn-2-yl)adenosine.



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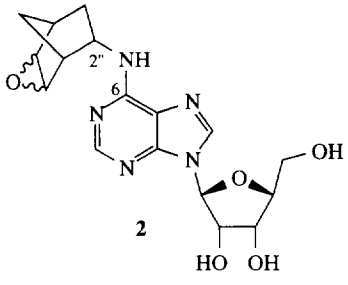


2

The design of these adenosine agonists has been supported by molecular modelling studies. A model of the A_1AdoR which was developed by Peet et. al.[4] postulated that xanthines bind to the receptor in a flipped and rotated

orientation with respect to adenosines. In this model, N1, N3, and N9 of adenosine were superimposed on N9, N3 and N1 of the xanthine. That approach places the N^6-amino hydrogen of adenosine close to the N7 hydrogen of the xanthine and, importantly, the N^6-substituent of adenosine coincides with the C8 substituent of the xanthine. Chiral 8-phenylisopropyl xanthine derivates were synthesised to test that model[4] and were found to possess the same stereoselectivity, though to a lower degree, as the corresponding N^6-(phenylisopropyl)adenosines. When this model was applied to ENX and the target molecules described in this communication, low energy conformations were found to access the same region.[8] Structures were optimised using the MOPAC semiempirical module (MNDO Hamiltonian) and an overlay of N9, N3, and N1 of ENX with N1, N3, and N9 of ENado was effected with the RMS fit procedure available in the InsightII program. Low energy conformations were identified by torsion about the rotatable bonds. Systematic 5° increments in the torsion angle of the C8-C2" bond of ENX as well as the C6-N^6 and N^6-C2" bonds of ENAdo was followed by evaluation of the total energy at each point. Conformations within 5 kcal/mol of the global minima were found which placed the epoxide oxygens of these compounds within 1.0 Å of each other (see Fig. 1a). This suggests that in the binding mode described by Peet et. al. the epoxide moieties should access a similar part of the of the A_1AdoR.

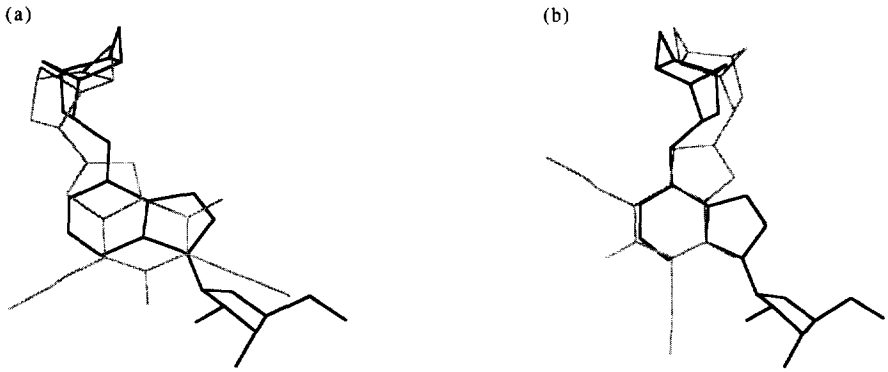


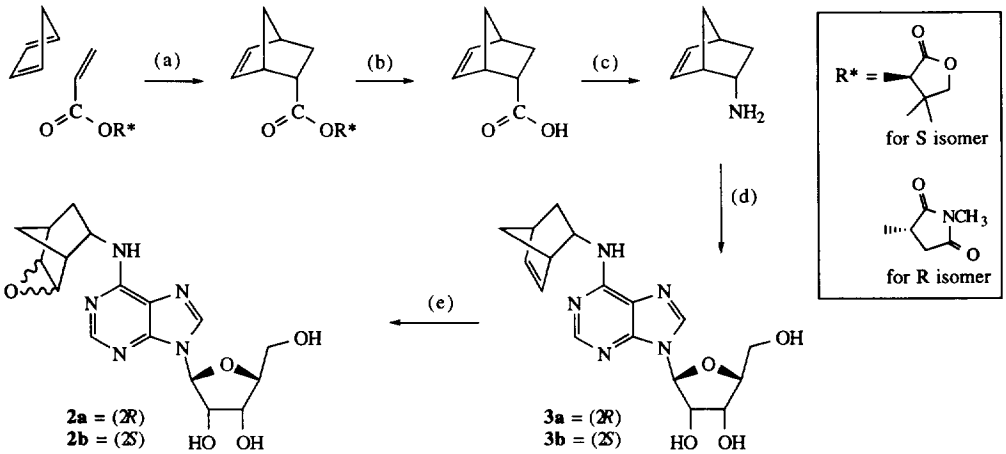
Fig 1 Superimposition of ENX (**1**) and ENAdo (**2**)

An alternative model of the A_1AdoR has been proposed by Dooley and Quinn[5] which also accounts for the structure/activity profile of N^6 adenosine and C8 xanthines. This model suggests that there are three binding domains which accommodate hydrophobic, aromatic and ribose moieties of adenosine agonists and antagonists. In particular, hydrophobic substituents in the N^6, C8, and C2 positions are thought to occupy the same region of the receptor. Application of this model to our compounds also identified low energy conformations which placed the epoxides of ENX and ENAdo within 1.0 Å of each other (see Fig. 1b).

N^6-substituted adenosines are generally prepared by alkylating an amine with 6-chloropurine riboside.[9] Initially (2*R*) and (2*S*)-*endo*-norborn-5-ene-2-carboxylic acids were prepared via the asymmetric Diels-Alder reactions described by Helmchen et al..[10,11] Enantiomeric excesses of 98 and 99% were obtained for the 2*R* and 2*S* isomers, respectively. Since the Curtius rearrangement is well known to proceed with retention of configuration,[12] an optimised version of this reaction[7] was used to convert these carboxylic acids to the corresponding amine hydrochlorides. Isolation of the free amines after treatment with base proceeded in low yield. However, this problem was avoided by liberating the amines in situ in the following step. The reaction of 6-chloropurine riboside with the amine hydrochlorides in the presence of an excess of triethylamine afforded good

yields of the N^6-substituted adenosines, **3a** and **3b**. Oxidation of the alkene moiety with dimethyldioxirane yielded the target molecules, **2a** and **2b**.[13] In contrast to recent reports of the oxidation by 5 equivalents of dimethyldioxirane of 2',3',5'-triacetyladenosine to the corresponding N1 oxide[14] and the oxidation of the 2',5'-di-O-trityl-N^6-trityl-, 5'-O-trityl-N^6-trityl- and the 3',5'-di-O-trityl-N^6-trityl derivatives of adenosine to the corresponding 8-oxoadenosine derivatives,[15] we observed no oxidation at the heterocyclic nitrogens under the reaction conditions used. This outcome is supported by both mass spectrometry and NMR data. The MS of the isolated product showed a molecular ion 16 mass units higher than the starting material, **3**, which indicated that it contained one extra oxygen. The ^1H NMR showed that the alkene protons of **3** (δ 5.97 and 6.35) had been replaced by a broad two proton doublet at δ 3.27, while the HMQC spectrum showed that these protons were attached to carbons at δ 48.3 and 50.7. Those chemical shifts are consistent with the presence of an epoxide and thus indicate that the additional oxygen observed in the mass spectra resulted from conversion of the alkene to an epoxide rather than oxidation at N1.

Scheme



(a) $TiCl_4$; (b) LiOH; (c) $SOCl_2$ then NaN_3, aq $(CH_3)_2CO$ then 2M HCl; (d) 6-chloropurine riboside, NEt_3, MeOH; (e) DMD[16]

The potency and efficacy fo the 2*R* and 2*S-endo* isomers (**2a** and **2b**) and cyclopentyladenosine were tested in DDT_1 MF-2 cells which express inhibitory A_1AdoR's for cAMP accumulation.[17] The procedures used for cell culturing and the determination of agonist mediated changes in cAMP accumulation have been described in detail previously.[18,19] The β-adrenergic agonist (-)isoproterenol (1 μM) stimulated cAMP accumulation 16-fold above the basal level. The 2*R* and 2*S-endo* isomers and CPA inhibited (-)isoproterenol stimulated cAMP accumulation in a concentration dependent manner. The concentration of CPA and the 2*S-endo* isomer that inhibited cAMP accumulation by 50% (EC_{50}) were similar and 10- to 12- fold more potent than the 2*R-endo* isomer (Table 1). This shows that the stereochemical orientation of the N^6-epoxynorborn-2-yl moiety plays a significant role in determining the potency of the compound for the A_1AdoR. In terms of efficacy, both the 2*R* and 2*S-endo* isomers gave the same maximal inhibition of cAMP accumulation as CPA indicating that they are full A_1AdoR agonists.

Table 1. A_1 agonist potency and maximal inhibition of (-)isoproterenol stimulated cAMP accumulation in DDT_1 MF-2 cells.

Compound	Inhibitory EC_{50} (nM)	% Maximal Inhibition
CPA	0.19 ± 0.29	80 ± 3
2a	10.0 ± 1.7	82 ± 4
2b	0.81 ± 0.16	81 ± 4

DDT_1 MF-2 cells were incubated with 1 μM (-)isoproternol and varying concentrations of the indicated compounds for 10 min at 37 °C. Basal and (-)isoproterenol stimulated cAMP accumulation values were 27 ± 7 and 421 ± 15 pmol cAMP formed per 10 min, respectively. Each value is the mean ± SE of 3 determinations performed in duplicate.

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13. **2a**: Dimethyldioxirane in acetone (38.4 mL, ~0.1 M, ~3.84 mmol) was added dropwise to a solution of N^6-(*endo*-norborn-5-en-2-yl)adenosine (0.69 g, 1.92 mmol) in dry methanol (40 mL) at 0 °C. The reaction mixture was stirred for 4 h at 0 °C and then 2 h at room temperature. The solvent was evaporated under reduced pressure and the crude product purified by column chromatography (on Merck Kieselgel 60) using EtOAc/MeOH/NH3 (90:10:1) as an eluent. Like fractions were combined and evaporated to afford a white foamy solid (0.54 g, 75 %); mp. 207–214 °C (dec.); $[\alpha]_D$ = -35.0 (MeOH); *^{1}H NMR*: δ (d^6 DMSO) 0.89–2.01 (m, 4H, H3″,7″), 2.44 (br s, 1H, H1″/4″), 2.81 (br s, 1H, H1″/4″), 3.23 (br s, 2H, H2″), 3.27 (br d, 2H, H5",6"), 3.63 (dd, 2H, H5'), 3.97 (q, 1H, H4'), 4.14 (t, 1H, H3'), 4.61 (br s, 1H, H2'), 5.20 (br s, 1H, OH), 5.39 (br s, 1H, OH), 5.45 (br s, 1H, OH), 5.89 (d, 1H, H1'), 7.87 (br s, 1H, NH), 8.24 (s, 1H, H2/8), 8.38 (s, 1H, H2/8); *^{13}C NMR*: δ 25.2, 31.2, 36.4, 38.5, 48.3, 49.0, 50.7, 61.6, 70.6, 73.4, 85.8, 87.9, 119.0, 139.7, 148.4, 152.2, 154.7; *HR MS* ($C_{17}H_{22}N_5O_5$) calc. 376.16208, found 376.16235.
2b: $[\alpha]_D$ = -35.0 (MeOH); *HR MS* ($C_{17}H_{22}N_5O_5$) calc. 376.16208, found 376.16264.
Storage of **2** at ambient temperature resulted in the appearance of an unidentified byproduct.
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