3′-Aminoadenosine-5′-uronamides: Discovery of the First Highly Selective Agonist at the Human Adenosine A_3 Receptor

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Received August 13, 2002

Abstract: Selective adenosine A_3 agonists have potential utility for the prevention of perioperative myocardial ischemic injury. Herein, we report on the discovery and synthesis of compound **7**. This amino nucleoside agonist possesses unprecedented levels of selectivity for the human adenosine A_3 receptor.

Patients undergoing cardiovascular surgery have an increased risk for a myocardial damaging ischemic event during or within a few days after the procedure.[1] In addition, noncardiovascular surgeries involving patients with risk factors for cardiovascular disease also carry an increased incidence of mortality and morbidity associated with ischemic injury.[2] Presently, there is no approved therapy for the prevention of myocardial ischemic injury despite the large number of exploratory approaches being investigated. One of these approaches involves a concept known as ischemic preconditioning (IP), which originated from the observation that repeated short episodes of ischemia will protect the heart from a subsequent longer, infarct-producing ischemic event.[3] Research around this phenomenon ultimately led to pharmacological mimics of IP, which includes the activation of cardiac adenosine receptors. Initial efforts focused on the preparation of selective A_1 receptor agonists.[4] Despite being cardioprotective, activation of the A_1 receptor leads to decreases in blood pressure and heart rate, effects deemed unacceptable in a surgical setting. More recently it has been shown that agonists at the A_3 receptor have cardioprotective properties without inducing hemodynamic effects in a rabbit model of ischemic injury.[5] Our goal was to discover a potent (<10 nM) agonist at the human A_3 receptor with >100-fold selectivity against the other human adenosine receptors (A_1, A_{2A}, A_{2B}) while possessing pharmaceutical properties suitable for iv administration. Herein, we report on the discovery of the amino nucleoside **7** (CP-608039), the first highly selective full agonist at the human adenosine A_3 receptor that in addition has water solubility suitable for developing an iv formulation.

Results. Since the first report on the isolation of the A_3 receptor in 1992, numerous agonists and antagonists have been reported for this target.[6] In the agonist arena, IB-MECA[6a] (**1**) and Cl-IB-MECA[6b] (**2**) have been used as prototypical selective A_3 agonists (Figure 1). However, although these analogues show selectivity against the rat receptors, there is poor correlation between species, an observation supported by the low receptor sequence homology.[7] Thus, as shown in Table 1, these compounds have only modest selectivity at the human receptors. Our discovery efforts began with the simplified MECA derivative **4**, which displayed potency and selectivity similar to those of **1**. Modifications to all positions of the ribose and adenine portions of compound **4** were achieved. These included atom substitutions in the base and sugar, as well as amide isosteres at C5′. Many of these changes gave disappointing results, leading to analogues with reduced potency, selectivity, or functional activity. One notable exception to this trend was the 3′-α-amino derivative **5**, which provided the desired level of selectivity at the expense of lower potency.

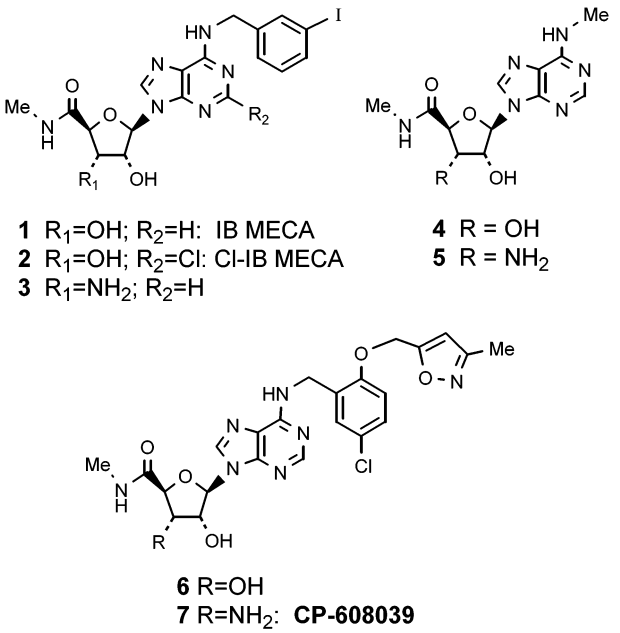

Figure 1. Compounds **1**−**7**.

Table 1. Binding to Human A_1 and A_3 Receptors[a]

compd	K_i[b] (nM) hA1	K_i[b] (nM) hA3	ratio A_1/A_3
1	20	4.4	4.5
2	99	14	6.9
3	4600[c]	72	64
4	65	4.8	13
5	23000	120	194
6	68[c]	1.6	42
7	7300	5.8	1260

[a] ^{125}I-ABA was used as the radioligand in these assays. [b] Values represent the average of at least three determinations unless otherwise noted. Average SEM is ±15%. [c] $n = 2$.

Compound **5**, as well as other 3′-amino analogues, was prepared as outlined in Scheme 1. Earlier strategies employing commercially available nucleosides (e.g., inosine) as starting materials proved to be unsatisfactory. The preparation of the required amino sugar in the "ribose" stereochemical configuration was readily achieved through an azide displacement of the triflate

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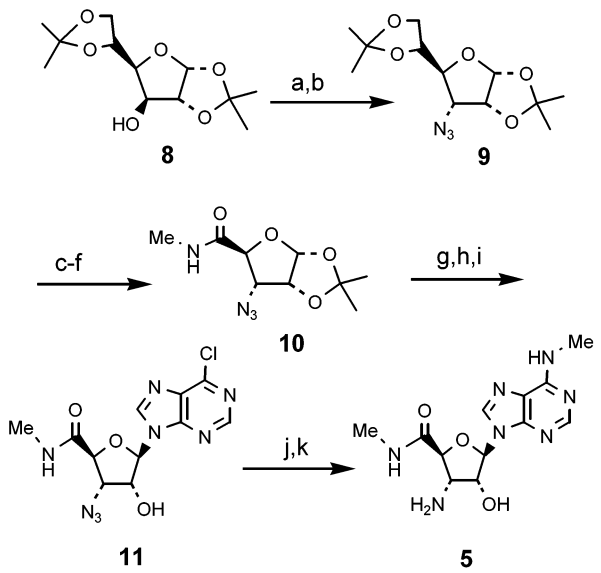
10.1021/jm0255724 CCC: $25.00
Published on Web 12/20/2002

Scheme 1[a]



[a] (a) Tf_2O, pyridine, CH_2Cl_2, −20 °C; (b) NaN_3, DMF, room temp; (c) HIO_4, THF−H_2O; (d) RuO_2, $NaIO_4$, $CHCl_3$, CH_3CN, H_2O; (e) $(COCl)_2$, CH_2Cl_2; (f) $MeNH_2$, CH_2Cl_2; (g) HOAc, Ac_2O, H_2SO_4; (h) TMS-6-chloropurine, TMSOTf, DCE, 60 °C; (i) Et_3N, MeOH; (j) $MeNH_2$, EtOH, 60 °C; (k) Ph_3P, NH_4OH, THF−H_2O.

derived from glucose diacetonide (**8**). Selective manipulation of the 5,6-acetonide provided the amide **10**. Hydrolysis of the remaining acetonide followed by Vorbrüggen[8] glycosidation using 6-chloro-9-trimethylsilylpurine afforded the intermediate **11**. Reaction of **11** with methylamine and reduction of the azide group with triphenylphosphine furnished the amino nucleoside **5**.

Attempts to recapture the high level of potency seen in **4** were made by elaborating the N-6 substituent. This region of the A_3 receptor is known to be highly tolerant to substitution,[6] and analogues were readily prepared from the late-stage intermediate **11** using the appropriate amine. Numerous incremental improvements ultimately led to the optimized structure **7**, which was chosen for clinical development. This compound binds to the human A_3 receptor with a K_i of 5.8 nM and possesses over 1000-fold selectivity versus the human A_1 receptor (Table 1). Even greater selectivity was observed over the human A_{2A} and A_{2B} receptors (K_i > 50 μM). Functional activity was measured in separate cell-based cAMP assays in which the human adenosine receptors were expressed. Compound **7** displayed full agonist activity at the hA_3 receptor, inhibiting the isoproterenol-stimulated increase in cAMP with an EC_{50} of 3.4 nM (Figure 2). Remarkably, greater than 1000-fold functional selectivity was also observed over the A_1, A_{2A}, and A_{2B} receptors. Further, the compound showed no antagonist activity at any of the receptors. In addition to enhancing A_3 selectivity, the 3′-amino group also endowed the molecule with favorable pharmaceutical properties. In particular, compound **7** has a pK_a of 6.25, resulting in acceptable aqueous solubility when formulated in acidic buffers (e.g., 2.5−5 mg/mL in 20 mM lactic acid).

For comparative purposes, the 3′-hydroxy analogue of **7** (compound **6**) and the 3′-amino analogue of IB-MECA (compound **3**) were synthesized and tested (Table 1). The most prominent attribute for **6** was its high level of potency, with an A_3 K_i of 1.6 nM. Compound **3** possessed only moderate levels of selectivity and potency. Clearly the 3′ amino group and N-6 substituent work in concert to provide the high levels of both A_3 binding affinity and selectivity seen in **7**.

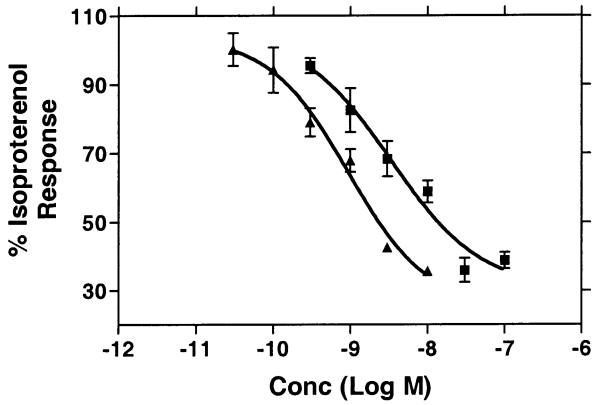


Figure 2. Concentration−response curve for the inhibition of isoproterenol-stimulated cAMP accumulation by **7** (■, EC_{50} = 3.4 nM) or IB-MECA (▲, EC_{50} = 1.0 nM) in HEK 293 cells expressing recombinant human A_3 receptors. Data are presented as the mean and SEM of three experiments, each run in duplicate. Maximal inhibition in this system is ∼70%.

In summary, the discovery of the first highly potent and selective human adenosine A_3 agonist has been achieved. Key to this discovery was the introduction of an amino group at the 3′ position of the nucleoside, which improved not only the selectivity but also the aqueous solubility. High water solubility is a prerequisite for the development of an agent for perioperative ischemic injury given the preferred parenteral route of administration. Further details of the pharmacology of compound **7** will be published elsewhere.

Supporting Information Available: Detailed chemistry and pharmacology experimental procedures and spectral data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM0255724