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The synthesis of highly potent, selective, and water-soluble agonists at the human adenosine A_3 receptor

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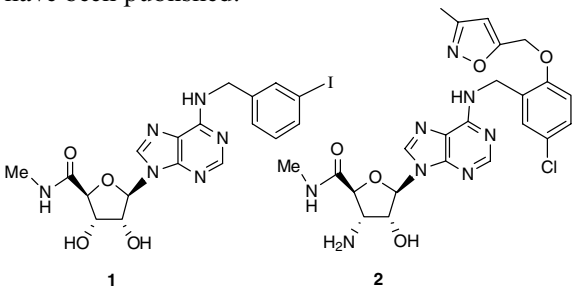
Abstract—Using a combination of parallel and directed synthesis, the discovery of a highly potent and selective series of adenosine A_3 agonists was achieved. High aqueous solubility, required for the intended parenteral route of administration, was achieved by the presence of one or two basic amine functional groups.

Adenosine is a ubiquitous neurotransmitter present in all cell types which exerts its actions through binding to four distinct G-protein-coupled receptors (A_1, A_{2A}, A_{2B}, and A_3). The elucidation of the biological functions of these receptors has been achieved in part by the availability of selective agonists and antagonists at the target proteins.[1] Not surprisingly, the state of understanding at each of the receptors accurately reflects the success of the medicinal chemistry efforts. Our interest in A_3 receptor agonists was initially driven not only by their cardioprotective properties,[2] but other potential indications as well, including cancer.[3] The pioneering work of Jacobson[4] has advanced our understanding of the functions of the A_3 receptor through the discovery of selective ligands, such as the highly studied agonist, IB-MECA (**1**). Building upon this work we have earlier reported on the identification of the first highly human selective A_3 agonist **2**.[5] Since that time, additional series of selective A_3 agonists have been published.[6]



1 **2**

Keywords: Adenosine; Agonist; A_3; Cardioprotection.
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Herein we describe the discovery of a related series of highly potent, selective, and water-soluble adenosine A_3 receptor agonists.

Our initial lead was the N-6 methyl uronamide analog **4a**. It possessed high potency for the A_3 receptor but only moderate selectivity over A_1. Our primary assay was for human A_1 and A_3 receptor binding. At the time of this work, there were no human A_{2A} or A_{2B} binding assays available, so functional assays were used to access activity at these receptors. In general, it was found that A_{2A} and A_{2B} functional activity tracked well with A_1 potency for these series of compounds.

Early on in the program it was found that the chemistry used to install the N-6 substituent on the adenosine template **3** was amenable to high-speed techniques. Consequently, a library of approximately 500 compounds was produced in a 96-well format. Several challenges emerged from this approach. At the time, parallel chemistry was in its infancy, and final products were not purified. Moreover, it was not potency but selectivity we were seeking, but we lacked the screening capacity to do full dose-downs on every analog. As a compromise, each analog was tested at two concentrations at both the A_3 (10, 100 nM) and A_1 (100, 1000 nM) receptors. Compounds that appeared particularly potent, and/or selective, were resynthesized for full characterization.

From these 500 analogs, only two emerged as particularly interesting (Table 1). The 3,5-dichloro benzyl analog **4b** was shown to be highly potent, but without improved

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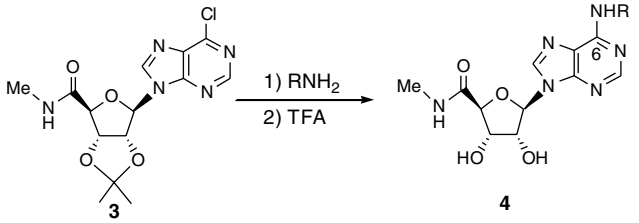
Table 1. Selected compounds identified from library of N-6 analogs

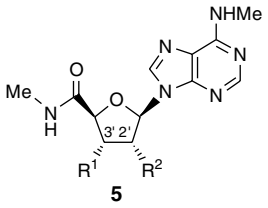


3 4

Compound	R	hA$_3$ K_i[a] (nM)	hA$_1$/hA$_3$[b]
1	3-Iodo benzyl	4.4 (±0.32)	4.5
4a	Methyl	4.8 (±0.61)	13
4b	3,5-Dichloro benzyl	2.5 (±0.21)	9.4
4c	2,5-Dimethoxy benzyl	3.9 (±0.3)	45

[a] Values represent means (±SEM) of at least three determinations unless noted.
[b] Selectivity versus the human A$_1$ receptor.

Table 2. 2′-3′ Analogs



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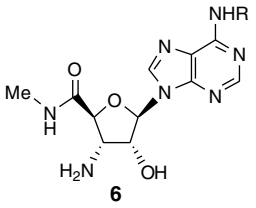
Compound	R^1	R^2	hA$_3$ K_i (nM)	hA$_1$/hA$_3$
5a	H	OH	59[a]	6
5b	OH	H	87[a]	12.6
5c	NHAc	OH	>3000	—
5d	NHSO$_2$Me	OH	>3000	—
5e	NHCONHEt	OH	>3000	—
5f	NH$_2$	OH	120 (±10.8)	194

[a] $n = 2$.

Table 3. N-6 Modifications in the 3′-amino series



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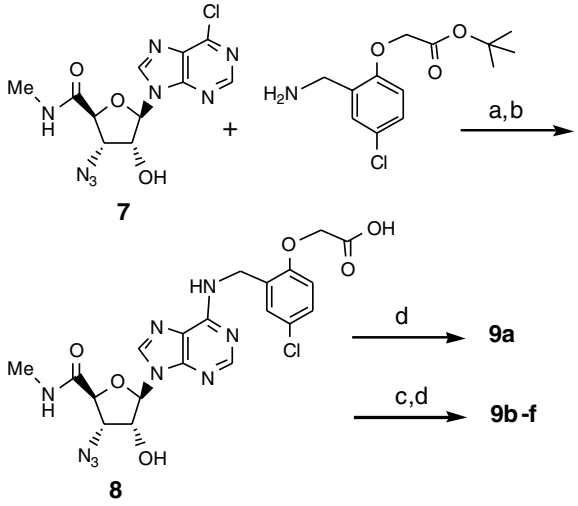
Compound	R	hA$_3$ K_i (nM)	hA$_1$/hA$_3$
6a	3,5-Dichloro benzyl	25 (±2.4)	148
6b	2,5-Dimethoxy benzyl	160 (±5.8)	294
6c	2-Methoxy-5-chloro benzyl	15 (±2.9)	317
6d	2-Benzyloxy-5-chloro benzyl	16 (±4.2)	691



Scheme 1. Reagents and conditions: (a) Et_3N, EtOH, 80°C; (b) TFA, CH_2Cl_2; (c) R^1R^2NH, EDCI, HOBt, DMF; (d) PPh_3, NH_4Cl, THF, H_2O.

selectivity, whereas, the 2,5-dimethoxy benzyl derivative **4c** had an A$_1$/A$_3$ ratio of 45. The benzyl substitution pattern in **4c** proved to be crucial later in the program (vide infra).

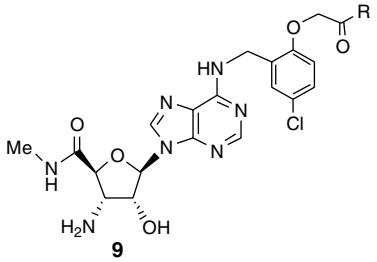
Concurrent with the library work, virtually all other regions of the lead were explored by directed synthesis. One area producing interesting SAR was the 2′,3′-diol (Table 2). Conventional wisdom suggested that these alcohols were critical for binding and agonist activity. Therefore, an emphasis was placed on analogs that maintained at least one hydrogen bond donor. Removal of either of the hydroxyl groups (**5a**, **5b**) did lead to a substantial loss of potency, although partial agonist activity was maintained. An amino series was envisioned to provide the necessary hydrogen bond, while allowing for additional substitution. In practice, no substitution on the amino group was allowed. Compounds **5c**–**e**, as well as *N*-alkyl derivatives (not shown) were all essentially inactive at the human A$_3$ receptor. Fortunately, the simple 3′-amine analog **5f** did show a dramatic improvement in selectivity, despite losing ~20-fold in potency.[5] Importantly, this analog maintained full agonist functional activity. Efforts were then directed back to the N-6 region in an attempt to recapture the single digit nanomolar potency of the diol lead.

The N-6 methyl group was first replaced with the amines identified in Table 1 (Table 3). The SAR appeared to be additive, with compounds **6a** and **6b** displaying improvements in potency and selectivity, respectively. Further modification of the 2,5 substitution pattern on the benzyl group revealed that large substituents were tolerated at the 2 position, whereas smaller groups, particularly halogen, were optimal at C5. This SAR is exemplified in compound **6d**, which served as a springboard to multiple subseries, including one that led to compound **2**.

One direction of research sought to replace the benzyl group in **6d** with more solubilizing functional groups.

To achieve this goal, the carboxylic acid **9a** was prepared as described in Scheme 1. The synthesis of compound **7** was described previously.[5] It was encouraging to see that this compound was active, which prompted further derivatization with bifunctional amines. These amides displayed excellent levels of potency and selectivity (Table 4).

Table 4. Amide analogs in the 3′-amino series



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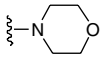
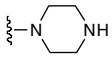
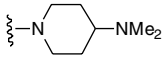
Compound	R	hA$_3$ K_i (nM)	hA$_1$/hA$_3$
9a	OH	130 (±12.4)	222
9b	NH_2	6.8 (±0.65)	526
9c		18 (±2.6)	720
9d		20 (±2.2)	700
9e		9.4 (±0.98)	445
9f		9.1 (±0.03)	456

Table 5. Functional activity[a] of compound **9e**

Compound	EC_{50} or % control at highest dose		
	hA$_{2A}$	hA$_{2B}$	hA$_3$
9e	16% at 3 μM	1% at 3 μM	8.1 nM

[a] Functional assays measured the increase of cAMP (A$_{2A}$ and A$_{2B}$) or the inhibition of isoproterenol-induced increase in cAMP (A$_3$) in HEK293 cells expressing the appropriate human receptor.

Analog **9e** was profiled further and was shown to be a potent, full agonist at the A$_3$ receptor, but functionally inactive at the A$_{2A}$ and A$_{2B}$ receptors (Table 5). It was negative in the in vitro micronucleus and Ames gene tox assays. As expected, **9e** has high aqueous solubility, particularly in buffered media (50–100 mg/mL in pH 4 citrate buffer). This compound was selected as a potential back-up to the earlier A$_3$ agonist candidate CP-608039, **2**.

In summary, a combination of both high speed and traditional medicinal chemistry techniques was used to identify a series of highly potent, selective, and water-soluble agonists at the human adenosine A$_3$ receptor.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.01.088.

References and notes

1. (a) Hess, S. *Expert Opin. Ther. Pat.* **2001**, *11*, 1533; (b) Volpini, R.; Costanzi, S.; Vittori, S.; Cristalli, G.; Klotz, K.-N. *Curr. Top. Med. Chem.* **2003**, *3*, 427; (c) Müller, C. E. *Curr. Top. Med. Chem.* **2003**, *3*, 445; (d) Jacobson, M. A. *Expert Opin. Ther. Pat.* **2002**, *12*, 489; (e) Hutchinson, S. A.; Scammells, P. J. *Curr. Pharm. Des.* **2004**, *10*, 2021; (f) Jacobson, K. A.; Tchilibon, S.; Joshi, B. V.; Gao, Z.-G. *Ann. Rep. Med. Chem.* **2003**, *38*, 121; (g) Yan, L.; Burbiel, J. C.; Maass, A.; Müller, C. E. *Expert Opin. Emer. Drugs* **2003**, *8*, 537; (h) Cristalli, G.; Lambertucci, C.; Taffi, S.; Vittori, S.; Volpini, R. *Curr. Top. Med. Chem.* **2003**, *3*, 387; (i) Soudijn, W.; van Wijngaarden, I.; IJzerman, A. P. *Curr. Top. Med. Chem.* **2003**, *3*, 355.
2. (a) Liang, B. T.; Jacobson, K. A. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6995; (b) Linden, J. *Mol. Pharmacol.* **2005**, *67*, 1385; (c) Headrick, J. P.; Peart, J. *Vasc. Pharmacol.* **2005**, *42*, 271; (d) Tracey, W. R.; Magee, W. P.; Oleynek, J. J.; Hill, R. J.; Smith, A. H.; Flynn, D. M.; Knight, D. R. *Am. J. Physiol.* **2003**, *285*(6, Pt. 2), H2780.
3. (a) Fishman, P.; Bar-Yehuda, S. *Curr. Top. Med. Chem.* **2003**, *3*, 463; (b) Fishman, P.; Bar-Yehuda, S.; Madi, L.; Cohn, I. *Anti-Cancer Drugs* **2002**, *13*, 437; (c) Duann, P.; Ho, T.-Y.; Desai, B. D.; Kapoian, T.; Cowen, D. S.; Lianos, E. A. *J. Invest. Med.* **2005**, *53*, 37; (d) Lee, E.-J.; Min, H.-Y.; Chung, H.-J.; Park, E.-J.; Shin, D.-H.; Jeong, L. S.; Lee, S. K. *Biochem. Pharmacol.* **2005**, *70*, 918; (e) Merighi, S.; Benini, A.; Mirandola, P.; Gessi, S.; Varani, K.; Leung, E.; Maclennan, S.; Borea, P. A. *J. Biol. Chem.* **2005**, *280*, 19516; (f) Baharav, E.; Bar-Yehuda, S.; Madi, L.; Silberman, D.; Rath-Wolfson, L.; Halpren, M.; Ochaion, A.; Weinberger, A.; Fishman, P. *J. Rheumatol.* **2005**, *32*, 469.
4. (a) Gallo-Rodriguez, C.; Ji, X. D.; Melman, N.; Siegman, B. D.; Sanders, L. H.; Orlina, J.; Fischer, B.; Pu, Q.; Olah, M. E.; van Galen, P. J.; Stiles, G. L.; Jacobson, K. A. *J. Med. Chem.* **1994**, *37*, 636; (b) Kim, H. O.; Ji, X. D.; Siddiqi, S. M.; Olah, M. E.; Stiles, G. L.; Jacobson, K. A. *J. Med. Chem.* **1994**, *37*, 3614; (c) Siddiqi, S. M.; Jacobson, K. A.; Esker, J. L.; Olah, M. E.; Ji, X. l.; Melman, N.; Tiwari, K. N.; Secrist, J. A., III; Schneller, S. W.; Cristalli, G.; Stiles, G. L.; Johnson, C. R.; IJzerman, A. P. *J. Med. Chem.* **1995**, *38*, 1174; (d) Jacobson, K. A.; Siddiqi, S. M.; Olah, M. E.; Ji, X.-d.; Melman, N.; Bellamkonda, K.; Meshulam, Y.; Stiles, G. L.; Kim, H. O. *J. Med. Chem.* **1995**, *38*, 1720; (e) Jiang, J.-I.; van Rhee, A. M.; Chang, L.; Patchornik, A.; Ji, X.-d.; Evans, P.; Melman, N.; Jacobson, K. A. *J. Med. Chem.* **1997**, *40*, 2596; (f) Kim, Y.-C.; Ji, X.-d.; Jacobson, K. A. *J. Med. Chem.* **1996**, *39*, 4142.
5. DeNinno, M. P.; Masamune, H.; Chenard, L. K.; DiRico, K. J.; Eller, C.; Etienne, J. B.; Tickner, J. E.; Kennedy, S. P.; Knight, D. R.; Kong, J.; Oleynek, J. J.; Tracey, W. R.; Hill, R. J. *J. Med. Chem.* **2003**, *46*, 353.
6. (a) Elzein, E.; Palle, V.; Wu, Y.; Maa, T.; Zeng, D.; Zablocki, J. *J. Med. Chem.* **2004**, *47*, 4766; (b) Baraldi, P. G.; Fruttarolo, F.; Tabrizi, M. A.; Romagnoli, R.; Preti, D.; Bovero, A.; Pineda de las Infantas, M. J.; Moorman, A.; Varani, K.; Borea, P. A. *J. Med. Chem.* **2004**, *47*, 5535; (c) Tchilibon, S.; Joshi, B. V.; Kim, S.-K.; Duong, H. T.; Gao, Z.-G.; Jacobson, K. A. *J. Med. Chem.* **2005**, *48*, 1745; (d) Van Rompaey, P.; Jacobson, K. A.; Gross, A. S.; Gao, Z.-G.; Van Calenbergh, S. *Bioorg. Med. Chem.* **2005**, *13*, 973.