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A new orally bioavailable dual adenosine A_{2B}/A_3 receptor antagonist with therapeutic potential

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Abstract—The synthesis and SAR of 5-heterocycle-substituted aminothiazole adenosine receptor antagonists is described. Several compounds show high affinity and selectivity for the A_{2B} and A_3 receptors. One compound (**5f**) shows good ADME properties in the rat and as such may be an important new compound in testing the current hypotheses proposing a therapeutic role for a dual A_{2B}/A_3 antagonist in allergic diseases.

Receptors for adenosine are currently of great interest as targets for therapeutic intervention due to their ubiquitous distribution throughout the body and their important modulatory effects on cell function.[1–6] Four distinct G-protein coupled receptors A_1, A_{2A}, A_{2B} and A_3 have been characterised and cloned,[7–13] and while A_1 and A_{2A} receptor pharmacology is well established, the role of the A_{2B} and A_3 receptors in human disease remains unclear. As a consequence, there has been an intensive effort to identify selective tools to facilitate pharmacological studies in vitro and in vivo.

We are particularly interested in the role of adenosine as a mediator in allergic asthma—an area which is currently receiving renewed attention.[5,14–24] The inhalation of adenosine causes bronchoconstriction in asthmatics, but not in normal subjects, and there is a significantly higher concentration of adenosine in the bronchoalveolar lavage fluid of asthma sufferers compared to normals.[25] However, the adenosine receptor subtype(s) involved in asthma and their potential role in the maintenance of the inflammatory response remains unclear. Current theory would suggest that an adenosine A_{2B} and/or A_3 receptor antagonist would be the appropriate molecular modifier to provide a therapy for asthmatics.[18,26–30] A_{2B} receptors mediate the synergistic effects of adenosine and allergen on human mast cells, which are believed to be involved in adenosine-induced bronchoconstriction in asthmatics. The role of the A_3 receptor is less clear, and a case could be argued for either an agonist or antagonist being beneficial in treating inflammation.[18,31]

We have previously described the design and synthesis of aminothiazoles as highly potent and selective adenosine A_3 receptor antagonists, which have been used to probe the role of adenosine in inflammation.[32] Investigation of the structure–activity relationship (SAR) profile of adenosine receptor antagonists revealed a pharmacophore hypothesis that enabled the production of compounds selective for the A_3 receptor.

As previously described,[32] we identified compound (**1**) (Fig. 1) as a lead from high throughput screening (HTS) of an in-house library against the A_3 receptor. Subsequent structural optimisation led to the

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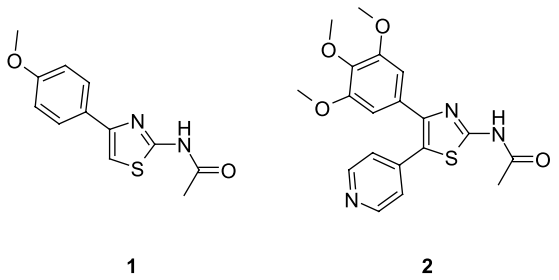


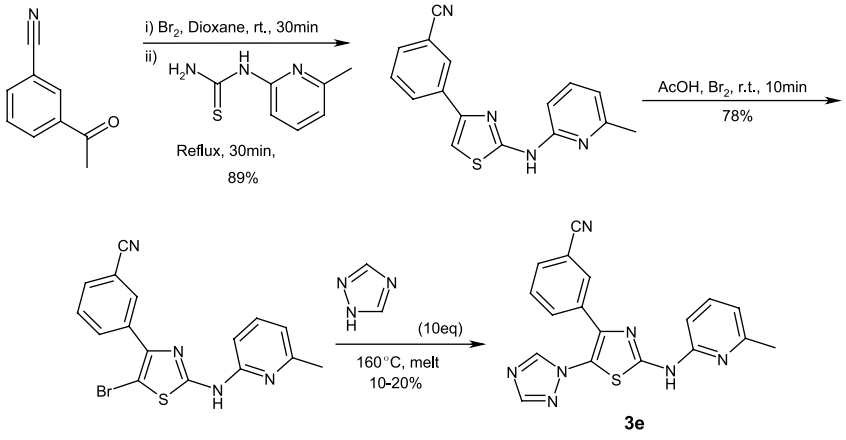
Figure 1. Structures of aminothiazole-based adenosine receptor antagonists.

synthesis of (**2**), an orally active, selective adenosine A_3 receptor antagonist. A series of 4-phenyl-5-pyridyl-aminothiazoles was synthesised, however, within this series it was not possible to obtain good potency at both A_{2B} and A_3 receptors whilst maintaining selectivity against A_1 and A_{2A}—which is expected to be necessary in order to achieve a compound with a low side effects profile. In this communication, we disclose the continuation of this work, which led to the synthesis and evaluation of a dual adenosine A_{2B}/A_3 receptor antagonist.

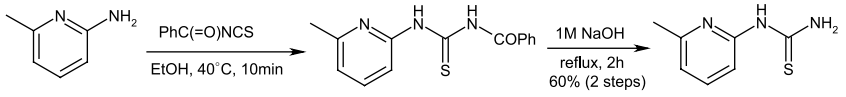
The aminothiazole moiety provides a scaffold allowing a range of different substitution patterns at positions 2, 4 and 5. We found that regardless of the groups at the 2- and 5-positions, the 3-cyanobenzene substituent at the 4-position generally bestowed the highest affinity for the A_{2B} and A_3 receptors, while giving some selectivity against A_1 and A_{2A}. This was in line with our previously described pharmacophore model.[32] During the course of this study, we expanded our SAR by varying the substitution at positions 2 and 5 on the ring with the aim of achieving greater selectivity and affinity for the A_{2B} and A_3 receptors.

As illustrated in Scheme 1, starting from commercially available acetophenones, bromination followed by Hantzsch-type cyclisation with thioureas (obtained by amine condensation with benzoylisothiocyanate followed by hydrolysis, as shown in Scheme 2) quickly yielded aminothiazoles unsubstituted at the 5-position. Treatment with bromine in acetic acid gave the 5-bromoaminothiazole as a stable solid. We then found that these compounds were able to undergo nucleophilic substitution on the bromine, which allowed replacement of the 5-pyridyl moiety present in **1** and **2** with smaller ring heterocycles such as imidazoles and triazoles, linked via a nitrogen–carbon bond. This is the first example, of which we are aware, of direct substitution of bromine on a 2-aminothiazole with aromatic nitrogen heterocycles. While the yield of the final step precluded the use of this route for large scale synthesis, by this process a large number of novel aminothiazole compounds was synthesised and screened for their affinity as adenosine receptor antagonists. The results observed with some of the more interesting compounds in our biological assays are shown in Table 1.[33]

We chose to replace the 2-aminoacetamide moiety of previously described compounds with pyridine derivatives. This was found to confer improved selectivity against the A_1 and A_{2A} receptors than had been previously seen (compare **3a** with **3b–f**). Our pharmacophore model suggested that a hydrogen bond acceptor on the amino group was important for A_{2B} and A_3 activity, and consistent with this, all pyridyl derivatives showed good affinity for these receptor subtypes. Increasing the basicity of the pyridyl nitrogen by incorporating an adjacent methyl group further improved both

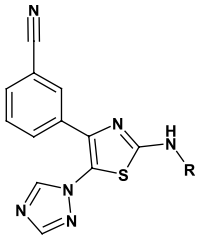
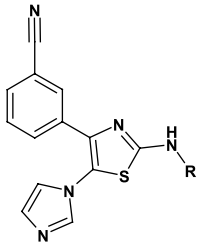
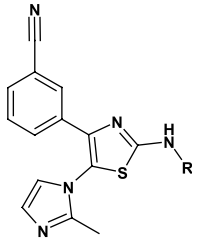


Scheme 1. Synthetic scheme for the preparation of compound **3e**. Other aminothiazoles are made by analogous routes.



Scheme 2. Example of a thiourea synthesis.

Table 1. In vitro activity of antagonists in human adenosine receptor assays[a]

Compounds		hA_1 K_i (nM)	hA_{2A} K_i(nM)	hA_{2B} K_B (nM)	hA_3 K_i (nM)
	**3a** R = Acetate	2840	5610	365	134
	3b R = 2-Py	1760	2020	53	24
	3c R = 3-Py	1530	2570	86	698
	3d R = 4-Py	3780	4160	62	304
	3e R = 6-Me-2Py	>10,000	>10,000	20	37
	3f R = Pyrazine	2090	3580	12	16
	**4c** R = 3-Py	560	2700	92	124
	4e R = 6-Me-2Py	8	>10,000	5	44
	4f R = Pyrazine	46	349	7	11
	**5e** R = 6-Me-2Py	224	175	10	419
	5f R = Pyrazine	197[b]	1670[c]	3[d]	10[e]

[a] The data represent the mean of at least two separate experiments, each performed in duplicate.
[b] SEM = ± 37 nM (n = 7).
[c] SEM = ± 460 nM (n = 6).
[d] SEM = ± 0.1 nM (n = 3).
[e] SEM = ± 0.3 nM (n = 6).

selectivity and potency (compare **3e** with **3b**). However, replacement of pyridine with the substantially less basic pyrazine (**3f**) was not notably detrimental to either potency or selectivity.

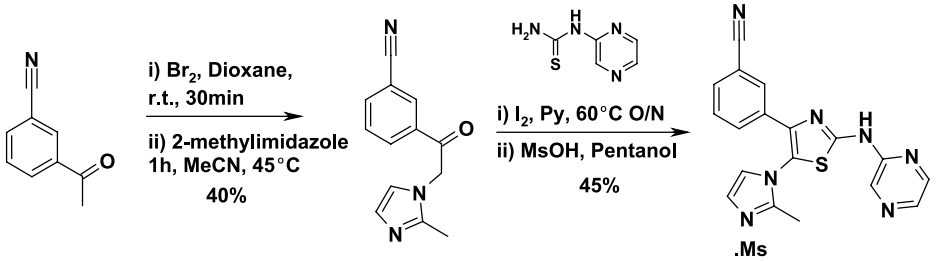
The SAR around the 5-position on the aminothiazole ring was rather less well defined, and affinity for the different receptors was very substrate dependent. However, in general it was seen that affinity to A_{2B} and A_3 receptors was maintained with triazole or (methyl) imidazole, but that the 5-triazole compounds showed greater selectivity against A_1 and A_{2A} receptors (compare **3f** with **5f** and **4f**; and **3e** with **4e** and **5e**).

Three of the compounds exemplified in Table 1 showed our desired selectivity and affinity profile at the adenosine receptors (**3e**, **3f** and **5f**), and were screened further for appropriate drug-like properties. In this regard, the mesylate salt of compound **5f**, 3-[5-(2-methylimidazol-1-yl)-2-(pyrazin-2-ylamino)-thiazol-4-yl]-benzonitrile, was found to be the superior compound. As well as being inactive against other receptor and enzyme targets in a broad screening panel, compound **5f** had a good in vivo pharmacokinetic profile in the rat, as shown in Table 2. The in vitro binding studies were carried out on human adenosine receptors, and it should be considered that the affinities and selectivity profile in rat recep-

Table 2. In vivo pharmacokinetics of compound **5f** (mesylate salt) in the Wistar rat

	$T_{1/2}$ (min)[a]	Clearance (mL/min/kg)[a]	V_{ss} (L/kg)[a]	C_{max} (μM)[c]	T_{max} (min)[c]	$T_{1/2}$ (min)[c]	BAV (%)[c]
Mean	29.0	94.4	3.02	0.56	39.3	191	30.2
SEM	3.13[b]	12.3[b]	0.64[b]	0.23[d]	15.0[d]	90.2[d]	7.78[d]

[a] Compound dosed iv in 2:1 PEG200/0.1 M phosphate buffer (pH 7.4) at 5 μmol/mL (2.28 mg/mL). Dosed at 1 mL/kg.
[b] n = 3.
[c] Compound dosed po in water (pH 4) at 15 μmol/mL (6.83 mg/mL). Dosed at 1 mL/kg.
[d] n = 7.



Scheme 3. Method for the large scale (up to 20 g) synthesis of **5f**.

tors may differ. However, we have previously shown with compounds in this series that the profile in rat is maintained in vivo.[32]

While the synthetic scheme detailed in Scheme 1 was appropriate for the small-scale production of a number of aminothiazole analogues, it was necessary to improve the process in order to synthesise enough material for this more advanced in vivo screening. To this end the synthesis detailed in Scheme 3 was elucidated. Thus, it was found that imidazole addition to 3-(2-bromoacetyl)benzonitrile occurred at 45 °C to give the cyclisation precursor. We found that direct bromination α- to the ketone followed by reaction with the thiourea was unsuccessful, however the use of iodine and the thiourea in pyridine did give the desired product in acceptable yield. We believe that this reaction probably proceeds via iodine-mediated oxidative dimerisation of the thiourea, followed by addition to the enolate. Salt formation was achieved in the normal way. By means of this route we were able to produce up to 20 g of final compound in three simple steps.

In conclusion, we have expanded upon the SAR of aminothiazoles as adenosine receptor antagonists. By means of novel chemistry, we have synthesised 5-heterocyclic substituted aminothiazoles, which have shown improved selectivity and affinity for the A_{2B} and A_3 receptors. One of these compounds, **5f**, has a good in vivo ADME profile when given orally in the rat, and as a potent, dual A_{2B} and A_3 antagonist, will be used to further develop the hypotheses regarding the role of these receptors in allergic disease.

References and notes

1. Jacobson, K. A.; Hoffmann, C.; Cattabeni, F.; Abbracchio, M. P. *Apoptosis* **1999**, *4*, 197–211.
2. Kaiser, S. M.; Quinn, R. J. *Drug Discovery Today* **1999**, *4*, 542.
3. Kowaluk, E. A. *Expert Opin. Invest. Drugs* **1998**, *7*, 535–543.
4. Mueller, C. E.; Stein, B. *Curr. Pharm. Des.* **1996**, *2*, 501.
5. Olah, M. E.; Stiles, G. L. *Annu. Rev. Pharmacol. Toxicol.* **1995**, *35*, 581–606.
6. Shryock, J. C.; Belardinelli, L. *Am. J. Cardiol.* **1997**, *79*, 2–10.
7. Furlong, T. J.; Pierce, R. D. S. L. A.; Shine, J. *Mol. Brain Res.* **1992**, *15*, 62.
8. Gurden, M. F.; Coates, J.; Ellis, F.; Evans, B.; Foster, M.; Hornby, E.; Kennedy, I.; Martin, D. P.; Strong, P. *Br. J. Pharmacol.* **1993**, *109*, 693.
9. Linden, J.; Jacobson, K. A. *Dev. Cardiovasc. Med.* **1998**, *209*, 1.
10. Murrison, E. M.; Goodson, M. R.; Edbrook, M. R.; Hase, T. *FEBS Lett.* **1996**, *384*, 243.
11. Olah, M. E.; Ren, H.; Stiles, G. L. *Arch. Int. Pharmacodyn. Ther.* **1995**, *329*, 135–150.
12. Palmer, T. M.; Stiles, G. L. *Neuropharmacology* **1995**, *34*, 683.
13. Sajjadi, F. G.; Firestein, G. S. *Biochem. Biophys. Acta* **1993**, *1179*, 105.
14. Coward, W. R.; Sagara, H.; Church, M. K. *Clin. Exp. Allergy* **1998**, *28*, 42–46.
15. Ezeamuzie, C. I.; Philips, E. *Br. J. Pharmacol.* **1999**, *127*, 188–194.
16. Feoktistov, I.; Biaggioni, I. *Drug Dev. Res.* **1997**, *39*, 333–336.
17. (a) Fozard, J. R. *Curr. Opin. Pharmacol.* **2003**, *3*, 264; (b) Fozard, J. R.; Hannon, J. P. *Pulm. Pharmacol. Ther.* **1999**, *12*, 111.
18. Fozard, J. R.; McCarthy, C. *Curr. Op. Inv. Drugs* **2002**, *3*, 69.
19. Holgate, S. T.; Cushley, M. J.; Church, M. K.; Hughes, P.; Mann, J. S. *Curr. Clin. Pract. Ser.* **1985**, *19*, 84–96.
20. Holgate, S. T. *Am. J. Phys.* **2002**, *282*, L167–L168.
21. Jacobson, K. A.; Moro, S.; Kim, Y. C.; Li, A. H. *Drug Dev. Res.* **1998**, *45*, 113–124.
22. Jacobson, K. A.; Jiang, J.-L.; Kim, Y.-C.; Karton, Y.; Van Rhee, A. M. International Patent WO9727177, 1997.
23. Jacobson, K. A. *Trends Pharmacol. Sci.* **1998**, *19*, 184–191.
24. Knight, D.; Zheng, X. Y.; Rocchini, C.; Jacobson, M. A.; Bai, T.; Walker, B. *J. Leukoc. Biol.* **1997**, *62*, 465.
25. Blackburn, M. R.; Young, H. W. J. *Drug Dev. Res.* **2003**, *58*, 472.
26. Baraldi, P. G.; Tabrizi, M. A.; Fruttarolo, F.; Bovero, A.; Avitabile, B.; Preti, D.; Romagnoli, R.; Merighi, S.; Gessi, S.; Varani, K.; Borea, P. A. *Drug Dev. Res.* **2003**, *58*, 315.
27. Fredholm, B. B. *Drug News Perspect.* **2003**, *16*, 283.
28. Ryzhov, S.; Goldstein, A. E.; Matafonov, A.; Zeng, D.; Biaggioni, I.; Feoktistov, I. *J. Immunol.* **2004**, *172*, 7726.
29. Zhong, H.; Shlykov, S. G.; Molina, J. G.; Sanborn, B. M.; Jacobson, M. A.; Tilley, S. L.; Blackburn, M. R. *J. Immunol.* **2003**, *171*, 338.
30. Zhong, H.; Belardinelli, L.; Maa, T.; Feoktistov, I.; Biaggioni, I.; Zeng, D. *Am. J. Resp. Cell Mol. Biol.* **2004**, *30*, 118.
31. Walker, B. A. M.; Jacobson, M. A.; Knight, D. A.; Salvatore, C. A.; Weir, T.; Zhou, D.; Bai, T. R. *Am. J. Resp. Cell Mol. Biol.* **1997**, *16*, 531.
32. Press, N. J.; Fozard, J. R.; Beer, D.; Haberthuer, S.; Heng, R.; Jones, K.; Schoeffter, P.; Tranter, P.; Keller, T. H. *Curr. Top. Med. Chem.* **2004**, *4*, 863.

33. Biological assay procedures. (i) A_1 receptor assay: A radioligand binding assay was used with the ligand [^{3}H]DPCPX and membranes from CHO cells transfected with the human A_1 receptor; (ii) A_{2A} receptor assay: A radioligand binding assay was used with the ligand [^{3}H]-ZM241385 and membranes from HEK 293 cells transfected with the human A_{2A} receptor; (iii) A_{2B} receptor assay: A reporter gene assay using CHO cells transfected both with the human A_{2B} receptor and a luciferase-expressing reporter plasmid was used. Activation of the A_{2B} receptor by NECA induces cAMP formation which, when bound to cAMP binding protein, elicits luciferase production from the reporter plasmid; (iv) A_3 receptor assay: A scintillation proximity assay was used with the ligand [^{125}I]-AB-MECA and CHO cells expressing the human adenosine A_3 receptor.