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Discovery of *N*-(5,6-diarylpyridin-2-yl)amide derivatives as potent and selective A_{2B} adenosine receptor antagonists

Paul Eastwood *, Jacob Gonzalez, Sergio Paredes, Arsenio Nueda, Teresa Domenech, Joan Alberti, Bernat Vidal

Almirall Research Center, Almirall S.A., Ctra. Laureà Miró 408, St. Feliu de Llobregat, E-08980 Barcelona, Spain

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ABSTRACT

The synthesis and SAR of a series of *N*-(5,6-diarylpyridin-2-yl)amide derivatives as potent A_{2B} adenosine receptor antagonists is described. Several compounds showed good selectivity versus other adenosine receptors. The potent and selective analogue **9** was shown to have good oral bioavailability in the rat.

The mediator adenosine has been linked to the pathophysiology of human asthma based on the observation that it triggers bronchoconstriction in asthmatics and the presence of high levels of this substance in the bronchoalveolar lavage fluid of asthmatics.[1–4] A_{2B} receptors are responsible for the adenosine-mediated release of several inflammatory cytokines from mast cells, airway and bronchial epithelial cells, fibroblasts, smooth muscle cells, intestinal epithelial cells, monocytes and dendritic cells.[4–7] These observations have led to the hypothesis that the A_{2B} receptor could be playing a major role as mediator of the effect of adenosine in human asthma.[3] Therefore, A_{2B} adenosine receptor antagonists have the potential to become a new pharmacological drug class for the treatment of this inflammatory condition.[8]

The likely similarity (Fig. 1), with respect to the pharmacophoric elements, of structures **1** (LAS38096), which pertains to a series of potent and selective A_{2B} adenosine receptor antagonists recently disclosed by our group,[9] and **2**, an orally active and selective A_3 receptor antagonist,[10] was considered. Both **1** and **2** possess a central heteroaromatic ring containing a hydrogen bond acceptor, two aromatic rings attached to the central core in an *ortho* arrangement, one of which is weakly basic in nature, and a hydrogen bond donating NH group. This led to the hypothesis that amides of type **3** would lead to novel series of antagonists. Herein is disclosed the synthesis and SAR of compounds of type **3** as potential A_{2B} adenosine receptor antagonists.

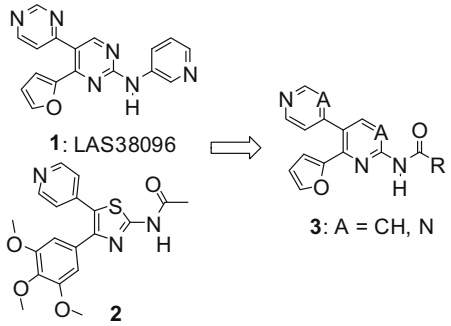

Figure 1. *N*-(5,6-Diarylpyridin-2-yl)amide derivatives.

Compound **5**, the acetamide analogue of **1**, was synthesized as shown in Scheme 1 by acetylation of the previously described intermediate **4**.[9] It was found in earlier studies that the 4-furyl-5-pyrimidinyl substitution pattern in compounds of type **1** led to a good balance of potency/selectivity with regard to A_{2B} versus other adenosine receptors. Thus, it was gratifying to find that **5** was a reasonably potent A_{2B} adenosine receptor antagonist that retained selectivity versus the other adenosine receptors (Table 1).[11,12]

Synthetic methods to access derivatives **6**–**14**, in which a pyridine nucleus is present as the central scaffold, are shown in Schemes 2–4 and biological results are given in Table 1.

Compound **6**, the pyridine equivalent of **5**, was found to have improved potency whilst retaining good selectivity. All other

* Corresponding author. Tel.: +34 933128610; fax: +34 933128635.
E-mail addresses: paulroberteastwood@yahoo.co.uk, paul.eastwood@almirall.com (P. Eastwood).

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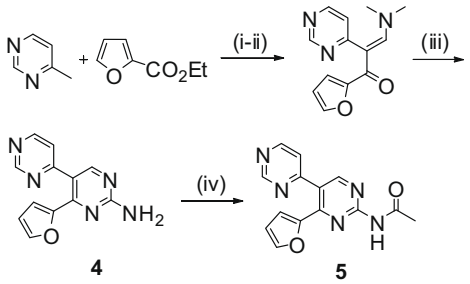
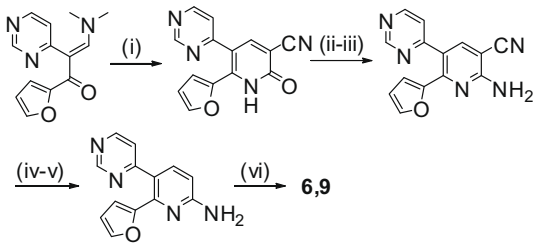
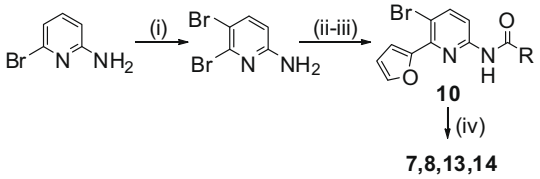


Scheme 1. Reagents and conditions: (i) LiHMDS, THF, 0 °C to rt, 94%; (ii) DMF–DMA, 100 °C, 87%; (iii) guanidine carbonate, K_2CO_3, DMF, 70 °C, 61%; (iv) CH_3COCl, pyridine, rt, 68%.



Scheme 2. Reagents and conditions: (i) 2-cyanoacetamide, NaOMe, DMF, 80 °C, 91%; (ii) $POCl_3$, 110 °C, 88%; (iii) satd NH_3 in EtOH, 80 °C, 76%; (iv) NaOH, EtOH, reflux, 99%; (v) Cu, quinoline, 205–215 °C, 58%; (vi) RCOCl, pyridine, 70 °C, 65–70%.



Scheme 3. Reagents and conditions: (i) NBS, DMF, rt, 86%; (ii) furan-2-ylboronic acid, $Pd(PPh_3)_4$, 2 M Na_2CO_3 (aq), toluene, MeOH, reflux, 32%; (iii) RCOCl, pyridine, 70 °C, 70–84%; (iv) ArB(pin), $PdCl_2$dppf·DCM, 2 M Cs_2CO_3 (aq), 1,4-dioxane, 85 °C, 40–65%.

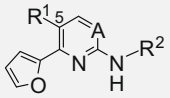
compounds described in this Letter possess the pyridyl moiety as the central scaffold. Analogues **7** and **8** show that by incorporation of a 4-pyridyl moiety at C-5 one arrives at very potent A_{2B} adenosine receptor antagonists, albeit with a certain loss of selectivity with respect to other adenosine receptors. Parallel studies utilizing a different central core ring system (unpublished results) showed that the optimal substituents for the amide moiety were acetyl and cyclopropyl and as such no further amide derivatives were prepared in this series. Incorporation of other non-basic substituents at C-5 (compounds **10** and **11**) gave potent but pan-adenosine receptor antagonists. Placement of an acidic tetrazole moiety at C-5 (compound **12**) abolished all potency. Derivative **14**, in which a fluorine atom was introduced in the *meta* position (with respect to the ring nitrogen) of the C-5 4-pyridyl moiety, had excellent potency and displayed an improved selectivity profile with respect to compound **8**. In general, it was found that placement of either a pyrimidin-4-yl (compound **9**) or a 3-fluoropyridin-4-yl radical (compound **14**) at C-5 of the pyridine core ring resulted in compounds with the best balance of potency and selectivity.

Attention was now turned to modifications at position C-6 with a focus on replacement of the furan moiety, a potential metabolic liability and toxicophore.[13] Synthetic methods are shown in Scheme 4 (compounds **15**–**33**) and the results are presented in Table 2.

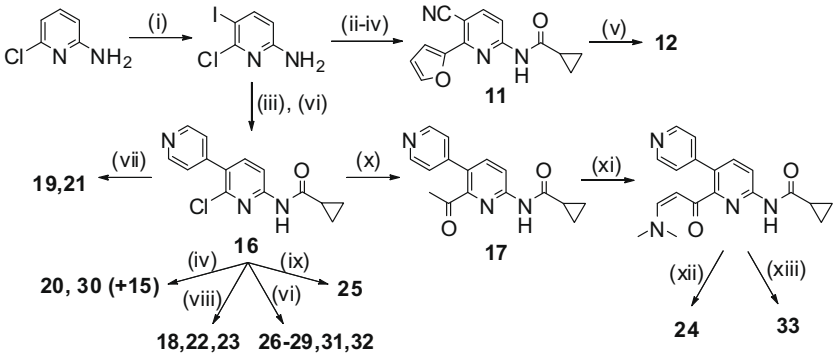
As expected from previous studies, the replacement of the furan moiety with non-aromatic substituents led to compounds which showed weaker antagonism at the A_{2B} receptor (compounds **15**–**17**). Placement of a methyl group on C-5 of the furan ring of **8**, which could potentially attenuate the formation of toxic metabolites derived from an unsubstituted furan ring, led to compound **18**, a potent A_{2B} adenosine receptor antagonist; however, the selectivity of this compound versus other adenosine receptors was unacceptable. Several other five-membered heterocyclic derivatives were prepared (compounds **19**–**25**). The oxazole derivative **21**, although not as potent as **8**, showed a similar selectivity profile. Other derivatives could not match the potency/selectivity profile of the furan derivative **8**. Regarding the placement of six-membered aromatic ring systems at C-6 it was discovered that the *ortho*-fluoro phenyl derivative **26** had a similar potency/selectivity profile to that of **8**. Once again all other derivatives synthesized could not match the potency/selectivity profile of furan **8**.

Selected compounds were profiled in an in vitro metabolism assay using hepatic microsomes (Table 3). Percentage turnover of the compounds was found to vary widely in both rat and human microsomes. Additionally, it was noted that in several instances non-oxidative metabolism was found to be occurring when assays were performed in the absence of the oxidative co-factor NAPDH. For example, in the case of compound **14**, a 99% turnover was noted upon incubation with rat microsomes both in the presence and absence of NADPH. Examination of the mass-spectral data from both studies revealed that the major metabolite was the corresponding 2-aminopyridine, the compound produced by the hydrolysis of the amide bond of **14**.

Table 1
Amide derivatives and modifications at C-5

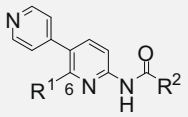


Compound	A	R_1	R_2	K_i (nM) or % inhibition of radioligand binding at a compound concentration of 1 μM)[12]			
				hA_{2B}	hA_{2A}	hA_1	hA_3
1	N	Pyrimidin-4-yl	Pyridin-3-yl	17 ± 4	40% ± 5	2821 ± 228	1043 ± 147
5	N	Pyrimidin-4-yl	C(=O)Me	380 ± 175	10% ± 2	2% ± 1	2% ± 1
6	CH	Pyrimidin-4-yl	C(=O)Me	47 ± 19	20% ± 4	7% ± 2	28% ± 2
7	CH	Pyridin-4-yl	C(=O)Me	7 ± 0	209 ± 45	358 ± 17	333 ± 50
8	CH	Pyridin-4-yl	C(=O)cyc-propyl	1 ± 0	30 ± 6	82 ± 19	1337 ± 383
9	CH	Pyrimidin-4-yl	C(=O)cyc-propyl	4 ± 1	239 ± 46	931 ± 121	3754 ± 866
10	CH	Br	C(=O)cyc-propyl	28 ± 2	35 ± 14	285 ± 79	617 ± 9
11	CH	C≡N	C(=O)cyc-propyl	41 ± 8	12 ± 2	289 ± 114	326 ± 7
12	CH	Tetrazol-5-yl	C(=O)cyc-propyl	23% ± 4	1638 ± 296	1% ± 1	8% ± 7
13	CH	3-Chloropyridin-4-yl	C(=O)cyc-propyl	5 ± 2	147 ± 18	250 ± 42	1866 ± 454
14	CH	3-Fluoropyridin-4-yl	C(=O)cyc-propyl	1 ± 0	150 ± 29	374 ± 22	11% ± 9



Scheme 4. Reagents and conditions: (i) NIS, DMF, rt, 86%; (ii) $Zn(CN)_2$, Pd_2dba_3, dppf, DMF, 120 °C, 80%; (iii) cyclopropylcarbonyl chloride, pyridine, 70 °C, 60–75%; (iv) $ArSnBu_3$, $Pd(PPh_3)_4$, xylenes, reflux, 53–86%; (v) NaN_3, NH_4Cl, DMF, 110 °C, 20%; (vi) ArB(pin) or $ArB(OH)_2$, $PdCl_2dppf{\cdot}DCM$, Cs_2CO_3 (aq), 1,4-dioxane, 80–100 °C, 22–67%; (vii) ArZnBr, $Pd(PPh_3)_4$, THF, reflux, 20–25%; (viii) ArH (oxazole or 2-methylfuran), $Pd(PPh_3)_4$, KOAc, DMA, 150 °C; (ix) pyrazole, Cs_2CO_3, DMF, 120 °C, 15%; (x) (1-ethoxyvinyl)tributylstannane, $Pd(PPh_3)_4$, xylenes, reflux then 2 M HCl (aq), rt, 90%; (xi) DMF–DMA, 100 °C, 99%; (xii) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 67%; (xiii) formamidine acetate, EtOH, toluene 115 °C, 42%.

Table 2
Modifications at C-6



Compound	R_1	R_2	K_i (nM) or % inhibition of radioligand binding at a compound concentration of 1 μM)[12]			
			hA_{2B}	hA_{2A}	hA_1	hA_3
8	Furan-2-yl	Cyclopropyl	1 ± 0	30 ± 6	82 ± 19	1337 ± 383
15	H	Cyclopropyl	29% ± 9	2% ± 1	8% ± 6	7% ± 5
16	Cl	Cyclopropyl	380 ± 133	7% ± 6	5% ± 3	14% ± 1
17	C(=O)Me	Cyclopropyl	3668 ± 882	2% ± 1	6% ± 4	2% ± 1
18	5-Methylfuran-2-yl	Cyclopropyl	5 ± 1	11 ± 3	25 ± 10	189 ± 23
19	Thiazol-2-yl	Cyclopropyl	42 ± 4	302 ± 25	914 ± 304	11% ± 9
20	Thiazol-5-yl	Cyclopropyl	78 ± 6	813 ± 138	320 ± 51	4851 ± 1522
21	Oxazol-2-yl	Cyclopropyl	32 ± 7	1359 ± 112	702 ± 245	22% ± 7
22	Oxazol-5-yl	Cyclopropyl	30 ± 9	635 ± 158	635 ± 158	5% ± 3
23	Oxazol-4-yl	Cyclopropyl	134 ± 34	1373 ± 397	1% ± 1	12% ± 5
24	1*H*-Pyrazol-3-yl	Cyclopropyl	202 ± 15	4% ± 1	3% ± 2	1% ± 0
25	1*H*-Pyrazol-1-yl	Cyclopropyl	72 ± 19	1440 ± 232	22% ± 9	2% ± 1
26	2-Fluorophenyl	Cyclopropyl	2 ± 1	61 ± 19	123 ± 41	817 ± 92
27	3-Fluorophenyl	Cyclopropyl	2 ± 0	57 ± 5	14 ± 3	715 ± 124
28	3-Fluorophenyl	Me	30 ± 11	286 ± 121	58 ± 4	471 ± 18
29	4-Fluorophenyl	Cyclopropyl	4 ± 1	39 ± 13	35 ± 3	27% ± 4
30	Pyridin-2-yl	Cyclopropyl	47 ± 24	862 ± 103	940 ± 182	2% ± 1
31	Pyridin-3-yl	Cyclopropyl	33 ± 3	1465 ± 568	21% ± 4	10% ± 4
32	Pyridin-4-yl	Cyclopropyl	14 ± 6	163 ± 56	338 ± 3	6% ± 4
33	Pyrimidin-4-yl	Cyclopropyl	208 ± 29	9% ± 1	3% ± 0	3% ± 1

Table 3
In vitro metabolism (hepatic microsomes)

Compound	% Turnover[a]	
	Rat	Human
9	27[b]	Not tested
14	99[b]	77[b]
21	27	14
26	100[b]	86[b]
30	12[b]	0

[a] % Turnover after a 30 min incubation period at 37 °C of a 5 μM solution of test compound with hepatic microsomes (1 mg/mL).
[b] Non-NADPH dependent metabolism observed.

Table 4
Pharmacokinetic profiles of derivatives **9**, **21** and **30** in rat (1 mg/kg iv)

Compound	$t_{½}$ terminal (h)	C_{max} (ng/mL)	Cl (L/h/kg)	$AUC_{0-\infty}$ (ng h/mL)	V_{ss} (L/kg)
9	0.5	538	3.8	267	1.6
21	0.9	761	1.9	533	1.6
30	0.5	822	2.1	485	1.0

The iv pharmacokinetic profiles in the rat of the compounds with the lowest in vitro metabolism (**9**, **21** and **30)** are presented in Table 4. Half lives in rat were short for all compounds tested as a result of moderate-to-high plasma clearance and moderate volumes of distribution.

An oral pharmacokinetic study conducted with **9** (10 mg/kg, rat, 0.1% tween 80 + 0.5% methylcellulose) showed that the compound was rapidly absorbed (T_{max} = 0.3 h) and had good bioavailability (F = 39%).

In summary, a new series of potent and selective A_{2B} adenosine receptor antagonists has been disclosed and preliminary data show that the compounds demonstrate oral bioavailability. In subsequent disclosures it will be shown how the further development of this series led ultimately to the selection of a clinical candidate.

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11. Results obtained with compounds **1** (previously reported—see Ref. 9), **5**–**7**, **10**, **28** and other related compounds in functional assays measuring cAMP levels in both human HEK293 cells (expressing the human A_{2B} receptor) and CHO cells (transfected with the mouse A_{2B} receptor) indicated that these compounds are acting as antagonists. Further details can be found in Ref. 9.
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