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LETTER

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Discovery of LAS101057: A Potent, Selective, and Orally Efficacious A_{2B} Adenosine Receptor Antagonist

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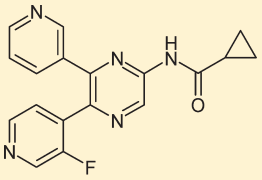
Supporting Information

ABSTRACT: The structure−activity relationships for a series of pyrazine-based A_{2B} adenosine receptor antagonists are described. From this work, LAS101057 (**17**), a potent, selective, and orally efficacious A_{2B} receptor antagonist, was identified as a clinical development candidate. LAS101057 inhibits agonist-induced IL-6 production in human fibroblasts and is active in an ovalbumin (OVA)-sensitized mouse model after oral administration, reducing airway hyperresponsiveness to methacholine, Th2 cytokine production, and OVA-specific IgE levels.

KEYWORDS: A_{2B} adenosine receptor antagonist, ovalbumin mouse model, clinical candidate



LAS101057 (**17**)

The purinergic, G protein-coupled receptor A_{2B} has a low affinity for the endogenous mediator adenosine.[1−3] Evidence published over the past decade has shown that the A_{2B} receptor mediates the release of inflammatory mediators from several cell types, including many which are relevant for the pathophysiology of asthma, such as mast cells, smooth muscle cells, airways epithelial cells, and fibroblasts.[4,5]

The A_{2B} receptor has a pro-inflammatory role in the gastrointestinal tract,[6] and it has been shown to contribute to the development of colitis in a murine A_{2B} receptor knockout model.[7] Some of the initial evidence obtained using A_{2B} receptor knockout mice suggested an anti-inflammatory role for this receptor.[8,9] However, subsequent studies do not entirely support this concept.[10−12] A_{2B} activates cystic fibrosis transmembrane regulator chloride channel-mediated liquid release in the lungs,[13] and it could play a role in severe watery infectious diarrhea through activation of a chloride secretory response.[14] A_{2B} receptor knockout mice have also shown that A_{2B} receptor prevents endothelial damage during inflammation and stimulates endothelial cell proliferation and capillary tube formation, mediating the release of the pro-angiogenic factors VEGF, bFGF, and IGF-1,[15] suggesting a possible role in tumorigenesis.[16] Expression of A_{2B} is upregulated in several types of cancer cells.[17,18]

A_{2B} adenosine antagonists that have been or are in clinical trials are the dual A_{2B}/A_3 antagonist QAF 805 and the selective A_{2B} antagonist CVT-6883.[19]

In previous disclosures,[20,21] it was revealed that appropriately substituted *N*-(5,6-diarylpyridin-2-yl)amides are potent and selective A_{2B} adenosine receptor antagonists. Although particular compounds from these series demonstrated good oral bioavailability in rat pharmacokinetic studies, in general all compounds tested presented rapid systemic clearance.

Herein are detailed further optimization efforts in a structurally related series which ultimately led to the discovery of a clinical candidate LAS101057 (**17**) intended for use in oral asthma therapy.

Studies conducted in parallel to those described above revealed that replacement of the central pyridine scaffold of compounds from the amide-based series by a pyrazine moiety gave compounds with good activity/selectivity profiles (Table 1).

The pharmacokinetic profiles of the pyrazines **1** and **2** in the rat were determined, and the results are given in Table 4. Despite showing excellent bioavailability and acceptable plasma levels, the acetamide analogue **2** suffered from a very short intravenous half-life ($t_{1/2}$ = 0.4 h). The corresponding cyclopropyl analogue **1**, in addition to showing complete oral bioavailability, demonstrated an improvement in the intravenous half-life ($t_{1/2}$ = 0.7 h) when compared to the acetamide derivative.

Encouraged by these preliminary findings, further exploration of the pyrazine series was undertaken. A range of amide derivatives were prepared (Table 1), and although several derivatives showed reasonable potency and selectivity, no compound showed an improvement upon the overall profile of the cyclopropyl derivative **1**.

Replacement of the two aromatic rings attached to the pyrazine central core was then investigated, keeping the cyclopropyl motif constant. Of particular interest was the replacement of the furan moiety, which is a potential metabolic liability and toxicophore.[22] Findings from parallel studies[20,21] led us to focus on specific replacements of these two rings which were most likely to deliver

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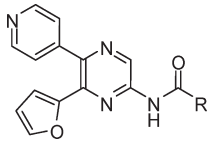
Table 1. SAR of Amide Substituent



		K_i (nM) or % inhibition of radioligand binding at indicated compound concentration (μM)			
compd	R	hA_{2B}	hA_{2A}	hA_1	hA_3
1	cyclopropyl	2.2 ± 0.2	44 ± 11	55 ± 17	398 ± 63
2	Me	6 ± 1	727 ± 143	454 ± 134	1563 ± 543
3	Et	3.5 ± 2	55 ± 21	42 ± 7	373 ± 118
4	i-Pr	10 ± 4	197 ± 62	18 ± 7	1920 ± 631
5	cyclobutyl	3 ± 1	140 ± 96	17 ± 6	4405 ± 7
6	cyclopentyl	13 ± 2	145 ± 23	27 ± 5	4076 ± 538
7	4-F-phenyl	63 ± 2	24 ± 5% (1)	1040 ± 454	17 ± 16% (1)
8	cyclopentylmethyl	12 ± 4	105 ± 5	98 ± 6	ND

a set of potent and selective A_{2B} receptor antagonists. Compounds synthesized and biological results are given in Table 2, and a representative synthesis, as exemplified by the preparation of **17** (LAS101057), is shown in Scheme 1.

Regioselective iodination of chloropyrazine (a) followed by a metal-catalyzed Stille coupling reaction with 3-fluoro-4-(tributylstannyl)pyridine[23] furnished the flexible intermediate (c). Amide formation followed by Suzuki coupling furnished compound **17** (LAS101057) in four steps in an overall yield of 21%.

As can be seen from the table, the majority of analogues prepared were found to potently displace radioligand binding to the A_{2B} receptor. Compounds with receptor selectivity profiles comparable to that of **1** could be obtained by replacement of the furan ring at C-6 with other aromatic nuclei, such as pyridyl, 2-fluorophenyl, and oxazolyl (compounds **10**−**15** and **17**−**23**), albeit at the expense of potency in the majority of cases. Nevertheless, desired levels of potency could be attained by the introduction of halogen atoms, particularly fluorine, into the pyridyl ring system at C-5 of the pyrazine. When combined, these particular changes at C-5 and C-6 led to a number of nonfuran containing compounds with excellent activity/selectivity profiles (compounds **17**−**23**).

The in vitro metabolism, permeability, and cytochrome P450 (CYP450) inhibition profiles of selected derivatives are given in Table 3. All compounds tested presented excellent Caco-2 permeabilities (P_{app} >30 × 10^{-6} cm/s) and were not found to be substrates of the P-glycoprotein efflux transporter (P-gp). Incubation of compounds with liver microsomes revealed a range of metabolic stabilities, although, in general, turnover was found to be low in both rat and human. N- and C-oxidation of the pyridine rings were found to be the major in vitro metabolic routes. In addition, minor amounts of metabolites derived from the hydrolysis of the amide bond were found in the liver microsome studies, although such compounds were not detected in plasma, blood, and liver subcellular fractions from different animal species and humans.

The majority of compounds showed no relevant inhibition of human CYP450 cytochromes CYP1A2, 2C9, and 2D6 (IC_{50} ≥ 25 μM). However, in certain cases, a moderate inhibition of CYP3A4 (IC_{50}: 1−25 μM) and CYP2C19 (IC_{50}: 1−5 μM) was observed. The potential for drug−drug interactions involving CYP2C19 was assessed by determination of the inhibition constant (K_i) for the most promising candidates (compounds **17**, K_i = 2.6 μM; **20**, K_i = 0.7 μM). These results indicated a potential risk of interaction in a clinical setting with drugs which are predominantly metabolized by CYP2C19.

The pharmacokinetic profiles of selected analogues were determined in the rat (Table 4). The compounds demonstrated moderate to excellent oral bioavailability in rat, and the combination of moderate clearance values and moderate volumes of distribution resulted in half-lives ranging from 0.8 to 1.6 h.

Based on the preliminary pharmacological and pharmacokinetic profile in the rat, compound **17** (LAS101057) was selected for further characterization. Additional pharmacokinetic studies (Table 5) showed that **17** had low plasma clearance in both dog and monkey with moderate to long half-lives: excellent bioavailability was observed in both species. Plasma protein binding (determined by ultrafiltration) was moderate in all species, accounting for 82%, 82%, 79%, and 89% in mouse, rat, dog, and human, respectively.

Since cAMP mediated signaling is one of the two main pathways elicited through activation of the A_{2B} receptor,[24] the antagonist activity of **17** was confirmed using a cell based functional assay measuring A_{2B} receptor-dependent intracellular cAMP levels using 5-(*N*-ethylcarboxamido)adenosine (NECA) as agonist.

In order to better interpret in vivo efficacy results obtained in the mouse, both human and mouse receptors were used for these assays. The results (IC_{50} (nM): human A_{2B} = 120 ± 21; mouse A_{2B} = 512 ± 67) indicated that **17** was active in inhibiting A_{2B} receptor-dependent cAMP signaling in both the human and mouse forms of the receptor and that the compound was approximately 4-fold more active in inhibiting signaling of the human receptor than that of the mouse receptor.

Given that the A_{2B} receptor is known to mediate the release of the pro-inflammatory cytokine IL-6 through a mechanism dependent on the cAMP/cAMP response element binding (CREB) signaling pathway,[24] it was decided to test the effect of **17** on NECA-induced IL-6 release in human primary dermal fibroblasts as an additional means of demonstrating blockade of A_{2B} receptor function. In this assay, **17** effected a concentration-dependent down-regulation of IL-6 production with a potency (67% ± 2 at 100 nM) that was in a similar range to that seen in both the A_{2B} receptor radioligand binding and cAMP assays.

The general selectivity of **17** was found to be excellent. When tested at a concentration of 10 μM in a diverse panel of more than 340 enzymes, receptors, channels, and transporters, **17** was found to be >400-fold selective.

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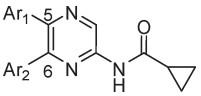
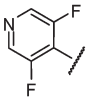
Table 2. SAR of Aromatic Substituents



			Ki (nM) or % inhibition of radioligand binding at indicated compound concentration (μM)			
Compound	Ar^1	Ar^2	hA_{2B}	hA_{2A}	hA_1	hA_3
9			2.2 ± 0	1.2 ± 0	11 ± 2	370 ± 79
10			11 ± 1	312 ± 64	312 ±178	18 ± 2 % (1)
11			25 ± 5	312 ± 192	30 ±8 % (1)	ND
12			32± 1	312± 54	33±7 % (1)	22±2 % (1)
13			1± 0	51± 6	25± 1	326 ± 44
14			56± 14	879±64	10±10% (1)	ND
15			43± 5	634±140	32±1% (1)	12 ± 10% (1)
16			29 ± 14	111 ± 17	2254± 6	9 ± 1 % (1)
17			24 ± 2	17±2% (2.5), 61 ± 2% (25)	56± 1.7% (10)	38±10% (10)
18			85 ±1	15% ± 5 (2.5)	23±10 (1)	5 ± 4 (1)
19			26 ±5	10±1% (2.5)	13±1% (1)	0 ± 0% (1)
20			11 ±2	1313±112	40±4% (1)	19 ± 7% (1)
21			0.6±0	101±33	130±44	8 ± 6% (1)
22			6±2	408±153	16% (1)	900 ± 474
23			19±1	18±9% (2.5)	18±1% (1)	9 ± 5% (1)

The efficacy of **17** was then determined in an ovalbumin (OVA)-sensitized mouse model: an in vivo functional model that recapitulates some of the pathological features of human asthma.[25] Relevant parameters that were determined from these studies included the airway hyperresponsiveness (AHR) to methacholine, numbers of inflammatory cells and levels of IL-4, IL-13, and γIFN present in bronchial alveolar lavage fluid (BAL), the extent of mucus accumulation, and plasma levels of OVA-specific IgE, total IgE, and IgG.

As shown in Figure 1, **17** reduced the increase of lung resistance induced by methacholine. LAS101057 (**17**) was active in preventing methacholine-induced AHR at 3 mg/kg, and at 10 mg/kg it inhibited AHR to methacholine to a level virtually equal to that seen with dexamethasone at 1 mg/kg.

Additionally, at 10 mg/kg, **17** reduced the levels of Th2 cytokines IL-4 (42 ± 12% inhibition) and IL-13 (44 ± 14% inhibition) in BAL but did not show any effect on the Th1 cytokine INFγ levels. **17** produced a slight but nonstatistically

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Table 3. Profiles of Selected Compounds[a]

			human CYP450 inhibition (IC_{50}, μM)[d]				
compd	% turnover[b] rat/human	P_{app} ($\times 10^{-6}$ cm/s)[c] A−B/B−A	1A2	2C9	2C19	2D6	3A4
1	46/18	36/26	5	5−25	<5	5−25	≈25
9	50/22	−	−	−	−	−	−
10	20/11	35/20	−	−	−	−	−
11	20/14	38/21	≈25	≈25	−	>25	5−25
12	15/12	34/22	>25	>25	−	5−25	1−5
17	13/5	37/23	>25	>25	2.6 (K_i)	>25	5−25
18	17/3	−	−	−	−	−	−
19	19/10	−	−	>25	≈1	>25	>25
20	4/2	33/22	>25	>25	0.7 (K_i)	>25	1−5
21	75/27	−	−	−	−	−	−
22	33/9	−	>25	−	≈1	−	>25
23	6/6	−	≈25	≈25	>25	>25	5−25

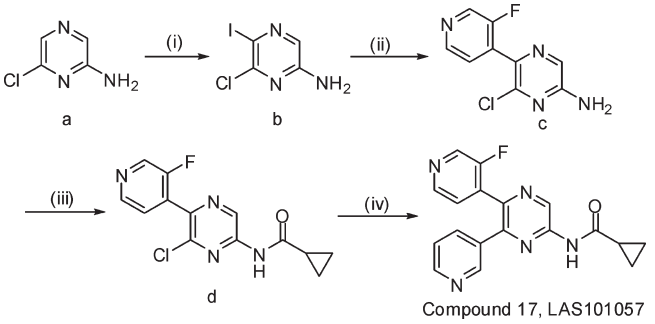
[a] −: Not tested. [b] % turnover after a 30 min incubation period at 37 °C of a 5 μM solution of test compound with hepatic microsomes (1 mg/mL). [c] Passive permeability through a CACO-2 monolayer determined using a 12.5 μM solution of test compound. [d] IC_{50} values were estimated by incubation of test compounds (1, 5, and 25 μM) with human liver microsomes using as selective CYP substrates 0.5 μM 7-ethoxyresorufin (CYP1A2), 10 μM diclofenac (CYP2C9), 50 μM *S*-mephenytoin (CYP2C19), 5 μM dextromethorphan (CYP2D6), and 50 μM testosterone (CYP3A4). Inhibition constants (K_i) for CYP2C19 were determined using different concentrations of *S*-mephenytoin (25−100 μM) and test substances (0.5−10 μM). Inhibition profiles obtained were consistent with a pure competitive model.

Table 4. Pharmacokinetic Properties of Selected Derivatives in Wistar Rats[a]

	i.v. (1 mg/kg)				p.o. (10 mg/kg)		
	$t_{1/2}$ (h)	AUC[b] (μg·h/mL)	Cl[c] (mL/(min kg))	V_{ss}[d] (L/kg)	C_{max} (μg/mL)	t_{max} (h)	F[e] (%)
1	0.7	933	18.3	1.1	2140	0.4	100
2	0.4	751	21.7	0.8	2294	1.1	93
10	1.0	1.31	12.8	0.74	0.87	2.5	100
11	0.9	0.98	17.3	0.93	3.75	0.6	100
17	0.8	0.92	18.3	1.2	7.11	0.25	100
19	1.6	1.11	15.0	1.4	2.88	0.6	68
20	1.2	0.68	24.5	1.3	1.46	2.5	53

[a] Mean values (n = 2). [b] Area under the curve. [c] Total plasma clearance. [d] Volume of distribution at steady state. [e] Bioavailability.

Scheme 1. Synthesis of 17 (LAS101057)[a]



[a] Reagents and conditions: (i) *N*-iodosuccinimide, DMSO/H_2O, 70%; (ii) 3-fluoro-4-(tributylstannyl)pyridine, $PdCl_2(Ph_3P)_2$, CuI, DMF, 150 °C, 57%; (iii) cyclopropanecarbonyl chloride, pyridine, 70 °C, 78%; (iv) pyridin-3-yl boronic acid, 2 M Cs_2CO_3, 1,1′-bis(diphenylphosphino)-ferrocene-palladium(II) dichloride ($PdCl_2$dppf), 1,4-dioxane, 90 °C, 70%.

significant reduction in mucus accumulation in the airway epithelia and demonstrated no effect in inhibiting lung tissue cell infiltrates (see Supporting Information).

Table 5. Pharmacokinetic Properties of 17 (LAS101057) in Preclinical Species[a]

	i.v.[b]						oral[c]		
species	$t_{1/2}$ (h)	AUC (μg·h/mL)	Cl (mL/(min kg))	V_{ss} (L/kg)	V_β (L/kg)	C_{max} (μg/mL)	t_{max} (h)	F (%)
mouse	−	−	−	−	−	17.4	0.25	−
monkey	3.8	3.4	3.4	0.4	0.8	5.9	3.0	100
dog	8.6	13.6	1.7	0.8	0.9	0.82	2.0	98

[a] −: Not tested. Mean values (n = 2). [b] iv administration: 1 mg/kg (male Beagle dogs) and 0.5 mg/kg (male Cynomolgus monkeys). [c] Oral administration: 10 mg/kg (female BALB/C mouse) and 1 mg/kg (male Beagle dogs and male Cynomolgus monkeys).

As shown in Table 6, **17** inhibited OVA-specific IgE production in a dose-dependent fashion, but neither the total IgE nor total IgG were affected.

Overall, the activity and efficacy data obtained with LAS101057 (**17**) indicates that this compound is active through selective inhibition of the A_{2B} receptor, adding to other pre-

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Table 6. Effect of 17 on Immunoglobulin Levels 24 h after the Last OVA Challenge[a]

	IgE OVA		total IgE		total IgG	
	plasma levels (μg/mL)	inhibition (%)	plasma levels (μg/mL)	inhibition (%)	plasma levels (μg/mL)	inhibition (%)
PBS	2.2 ± 0 02[b]		0.7 ± 0.04[b]		275 ± 13[b]	
OVA	330 ± 104		13 ± 0.8		613 ± 22	
LAS101057 3 mg/kg	165 ± 52	50 ± 16	11 ± 0.8	12 ± 8	571 ± 29	12 ± 8
LAS101057 10 mg/kg	63 ± 31[b]	81 ± 9	11 ± 1	7 ± 5	632 ± 29	0 ± 9
dexamethasone 1 mg/kg	68 ± 21[b]	80 ± 6	8 ± 0.8[b]	41 ± 7	590 ± 18	7 ± 5

[a] Data represent mean ± SEM of 6-8 animals per group. [b] $p < 0.05$ Comparison with t-student test versus OVA group.

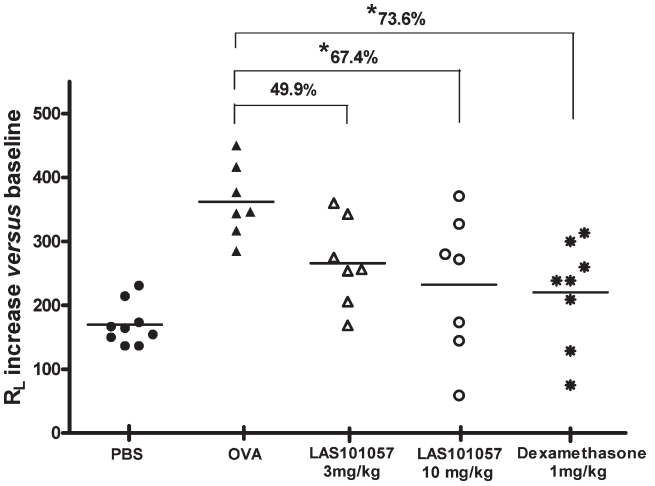

Figure 1. Effect of **17** on airway hyperresponsiveness to methacholine 24 h after the last OVA challenge. Data represent mean; numbers represent the inhibition percentages compared to the OVA group. *$p <$ 0.05, one way ANOVA with Dunnett's post-test.

clinical studies which have shown that the anti-inflammatory effects mediated by selective blockade of the A_{2B} receptor may be beneficial in a range of disease states.[2,3]

In conclusion, herein is reported the discovery of **17**, a novel potent, selective, and orally efficacious A_{2B} antagonist. LAS101057 (**17**) displays excellent potency in both mechanistic and cell based functional assays and exhibits good in vivo activity in the OVA-sensitized mouse model of asthma. Antagonism of the A_{2B} receptor is expected to have a beneficial effect on asthma therapy, and as such, LAS101057, after successfully undergoing further preclinical safety and toxicology characterization, was subsequently advanced into a phase I, open-label, dose-escalation study in healthy subjects.

■ ASSOCIATED CONTENT

Supporting Information. Detailed experimental procedures for the synthesis of compounds **1**−**23**, in vitro assay protocols, procedures for the pharmacokinetic studies, protocol for the ovalbumin (OVA)-sensitized mouse model, and full selectivity profile. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

Bernat Vidal was the chemistry project leader, the main inventor of the chemical series, the person directly responsible for the design and synthesis of LAS101057, and the key decision maker in the selection of LAS101057. Arsenio Nueda was the biology project leader, the designer and coordinator of the experiments performed, and the key decision maker in the selection of LAS101057. Teresa Domenech was the person responsible for performing radioligand binding assays. María Isabel Loza and María Isabel Cadavid set up and provided data on the cAMP in vitro assays and performed some of the radioligand binding assays. Neus Prats was responsible for the histology studies. Núria Godessart and Mercè Pont designed the experiments and performed experimental work on the human fibroblasts studies. Joan Albertí was mainly responsible for the selection, prioritization, and interpretation of in vitro and in vitro ADME studies and for performing dog and monkey studies. Mónica Córdoba performed in vitro metabolism, permeability, and CYP studies. Raquel Fernández performed pharmacokinetic studies in rats. Montserrat Miralpeix and Mònica Aparici designed experiments and performed experimental work on OVA efficacy studies. Paul Eastwood, Cristina Esteve, Jacob González, Silvia Fonquerna, Josep Aiguadé, and Inés Carranco explored the structure−activity relationships, optimization of substituents, optimization of synthetic routes and yields, and identification and characterization of compounds.

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■ REFERENCES

(1) Polosa, R. Adenosine-receptor subtypes: their relevance to adenosine-mediated responses in asthma and chronic obstructive pulmonary disease. *Eur. Respir. J.* **2002**, *20*, 488–496.

(2) Fredholm, B. B.; Irenius, E.; Kull, B.; Schulte, G. Comparison of the potency of adenosine as an agonist at human adenosine receptors expressed in Chinese hamster ovary cells. *Biochem. Pharmacol.* **2001**, *61*, 443–448.

(3) Reviewed in: Haskó, G.; Linden, J.; Cronstein, B.; Pacher, P. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. *Nat. Rev. Drug Discov.* **2008**, *7*, 759–770.

(4) Holgate, S. T. The Quintiles Prize Lecture 2004. The identification of the adenosine A_{2B} receptor as a novel therapeutic target in asthma. *Br. J. Pharmacol.* **2005**, *145*, 1009–1015.

(5) Polosa, R.; Blackburn, M. Adenosine receptors as targets for therapeutic intervention in asthma and chronic obstructive pulmonary disease. *Trends Pharmacol. Sci.* **2009**, *30*, 528–535.

(6) Sitaraman, S. V.; Merlin, D.; Wang, L.; Wong, M.; Gewirtz, A. T.; Si-Tahar, M.; Madara, J. L. Neutrophil-epithelial crosstalk at the intestinal lumenal surface mediated by reciprocal secretion of adenosine and IL-6. *J. Clin. Invest.* **2001**, *107*, 861–869.

(7) Kolachala, V. L.; Vijay-Kumar, M.; Dalmasso, G.; Yang, D.; Linden, J.; Wang, L.; Gewirtz, A.; Ravid, K.; Merlin, D.; Sitaraman, S. V. A2B adenosine receptor gene deletion attenuates murine colitis. *Gastroenterology* **2008**, *135* (3), 861–870.

(8) Yang, D.; Zhang, Y.; Nguyen, H. G.; Koupenova, M.; Chauhan, A. K.; Makitalo, M.; Jones, M. R.; St Hilaire, C.; Seldin, D. C.; Toselli, P.; Lamperti, E.; Schreiber, B. M.; Gavras, H.; Wagner, D. D.; Ravid, K. The A_{2B} adenosine receptor protects against inflammation and excessive vascular adhesion. *J. Clin. Invest.* **2006**, *116* (7), 1913–1923.

(9) Hua, X.; Kovarova, M.; Chason, K. D.; Nguyen, M.; Koller, B. H.; Tilley, S. L. Enhanced mast cell activation in mice deficient in the A_{2B} adenosine receptor. *J. Exp. Med.* **2007**, *204* (1), 117–128.

(10) Ryzhov, S.; Zaynagetdinov, R.; Goldstein, A. E.; Novitskiy, S. V.; Dikov, M. M.; Blackburn, M. R.; Biaggioni, I.; Feoktistov, I. Effect of A_{2B} adenosine receptor gene ablation on proinflammatory adenosine signaling in mast cells. *J. Immunol.* **2008**, *180*, 7211–7220.

(11) Ryzhov, S.; Zaynagetdinov, R.; Goldstein, A. E.; Novitskiy, S.; Blackburn, M. R.; Biaggioni, I.; Feoktistov, I. Effect of A_{2B} adenosine receptor gene ablation on adenosine-dependent regulation of proinflammtory cytokines. *J. Pharmacol. Exp. Ther.* **2008**, *324*, 694–700.

(12) Zaynagetdinov, R.; Ryzhov, S.; Goldstein, A. E.; Huiyong, Y.; Novitskiy, S. V.; Goleniewska, K.; Polosukhin, V. V.; Newcomb, D. C.; Mitchell, D.; Morschl, E.; Zhou, Y.; Blackburn, M. R.; Peebles, R. S.; Biaggioni, I.; Feoktistov, I. Attenuation of chronic pulmonary inflammation in A_{2B} adenosine receptor knockout mice. *Am. J. Respir. Cell Mol. Biol.* **2010**, *42*, 564–571.

(13) Lazarowski, E. R.; Boucher, R. C. Purinergic receptors in airway epithelia. *Curr. Opin. Pharmacol.* **2009**, *3*, 262–267.

(14) Crane, J. K.; Olson, R. A.; Jones, H. M.; Duffey, M. E. Release of ATP during host cell killing by enteropathogenic E. coli and its role as a secretory mediator. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2002**, *283*, G74–G86.

(15) Haskó, G.; Csóka, B.; Németh, Z. H.; Vizi, E. S.; Pacher, P. A_{2B} adenosine receptors in immunity and inflammation. *Trends Immunol.* **2009**, *30*, 263–70.

(16) Ryzhov, S.; Novitskiy, S. V.; Zaynagetdinov, R.; Goldstein, A. E.; Carbone, D. P.; Biaggioni, I.; Dikov, M. M.; Feoktistov, I. Host A_{2B} adenosine receptors promote carcinoma growth. *Neoplasia* **2008**, *10*, 987–995.

(17) Li, S.; Huang, S.; Peng, S. B. Overexpression of G protein-coupled receptors in cancer cells: involvement in tumor progression. *Int. J. Oncol.* **2005**, *27*, 1329–1339.

(18) Ma, D. F.; Kondo, T.; Nakazawa, T.; Niu, D. F.; Mochizuki, K.; Kawasaki, T.; Yamane, T.; Katoh, R. Hypoxia-inducible adenosine A_{2B} receptor modulates proliferation of colon carcinoma cells. *Hum. Pathol.* **2010**, PMID: 20619442.

(19) Polosa, R.; Blackburn, M. R. Adenosine receptors as targets for therapeutic intervention in asthma and chronic obstructive pulmonary disease. *Trends Pharmacol. Sci.* **2009**, *30* (10), 528–535.

(20) Eastwood, P.; Gonzalez, J.; Paredes, S.; Fonquerna, S.; Cardús, A.; Alonso, J. A.; Nueda, A.; Domenech, T.; Reinoso, R. F.; Vidal, B. Discovery of potent and selective bicyclic A_{2B} adenosine receptor antagonists via bioisosteric amide replacement. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1634–1637.

(21) Eastwood, P.; Gonzalez, J.; Paredes, S.; Nueda, A.; Domenech, T.; Alberti, J.; Vidal, B. Discovery of N-(5,6-diarylpyridin-2-yl)amide derivatives as potent and selective A_{2B} adenosine receptor antagonists. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1697–1700.

(22) Kalgutkar, A. S.; Gardner, I.; Obach, R. S.; Shaffer, C. L.; Christopher, L.; Callegari, E.; Henne, K. R.; Mutlib, A. E.; Dalvie, D. K.; Lee, J. S.; Nakai, Y.; O'Donnell, J. P.; Boer, J.; Harriman, S. P. A Comprehensive Listing of Bioactivation Pathways of Organic Functional Groups. *Curr. Drug Metab.* **2005**, *6*, 161–225.

(23) Prepared according to EP1104754 (Reference Example 197).

(24) Murakami, S.; Terakura, M.; Kamatani, T.; Hashikawa, T.; Saho, T.; Shimabukuro, Y.; Okada, H. Adenosine regulates the production of interleukin-6 by human gingival fibroblasts via cyclic AMP/protein kinase A pathway. *J. Periodontal Res.* **2000**, *35*, 93–101.

(25) Lloyd, C. M.; Gonzalo, J. A.; Nguyen, T.; Delaney, T.; Tian, J.; Oettgen, H.; Coyle, A. J.; Gutierrez-Ramos, J. C. Resolution of bronchial hyperresponsiveness and pulmonary inflammation is associated with IL-3 and tissue leukocyte apoptosis. *J. Immunol.* **2001**, *166*, 2033–2040.