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New pyrrolopyrimidin-6-yl benzenesulfonamides: Potent A_{2B} adenosine receptor antagonists

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Abstract—A new series of 4-(1,3-dialkyl-2,4-dioxo-2,3,4,5-tetrahydro-1*H*-pyrrolo[3,2-*d*]pyrimidin-6-yl)benzenesulfonamides has been identified as potent A_{2B} adenosine receptor antagonists. The products have been evaluated for their binding affinities for the human A_{2B}, A_1 and A_3 adenosine receptors. 6-(4-{[4-(4-Bromobenzyl)piperazin-1-yl]sulfonyl}phenyl)-1,3-dimethyl-1*H*-pyrrolo[3,2-*d*]pyrimidine-2,4(3*H*,5*H*)-dione (**16**) showed a high affinity for the A_{2B} adenosine receptor (IC_{50} = 1 nM) and selectivity (A_1: 183x; A_3: 12660x). Synthesis and SAR of this novel class of compounds showing improved absorption properties is presented herein.

Adenosine is an endogenous nucleoside that is normally increased under conditions of hypoxia or high metabolism typically occurring in pathological or stressful situations. Adenosine interacts with four G-protein-coupled membrane receptors, A_1, A_{2A}, A_{2B} and A_3, with different affinities. A_1 and A_{2A} are high affinity receptors for adenosine with affinities in the range of 0.01 μM, while A_{2B} and A_3 are low affinity receptors, with affinities in the range of 5–10 μM.[1]

Concentration of extracellular adenosine in normal tissues is estimated to be below 1 μM, with levels increasing to as much as 100 μM during inflammatory or ischaemic conditions.[2] Thus, while tissue expression of all four adenosine receptors is ubiquitous, different affinities for adenosine indicate that A_{2B} and A_3 receptors would not be constitutively activated in normal conditions.

There is a large body of evidence linking the A_{2B} receptor to the pathophysiology of asthma:[3]

- Adenosine is present in the bronchoalveolar lavage (BAL) of asthmatic subjects at concentrations up to 4-fold higher than in normal subjects (193 vs 60 μM)[4]
- It is well documented that the A_{2B} receptor has likely roles in mast cell degranulation and in the stimulation of IL6, IL8, IL4/IL13, IL19 and MCP-1 secretion pathways both in mast cells and in other cell types like lung fibroblasts and bronchial smooth muscle cells.[5]

The hypothesis that A_{2B} receptor antagonism mediates the antiasthmatic properties of theophylline, a clinically proven antiasthmatic with severe safety limitations due to poor selectivity,[6] together with the recent description of A_{2B} receptors on human airway smooth muscle cells mediating cytokine and chemokine release provides firm basis to recognize A_{2B} antagonists as potential novel anti-asthma drugs.[7]

As a first approach to design novel A_{2B} adenosine receptor antagonists, we developed a series of pyrrolopyrimidines[8] that led to the discovery of compound (**1**)

Keywords: A_{2B} adenosine receptor antagonists; Asthma; Pyrrolopyrimidine; Sulfonamides.
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(Fig. 1) showing good potency and selectivity (against A_{2A} and A_3 receptors) but modest selectivity versus A_1 adenosine receptor. Additionally, amide metabolic liability together with a low solubility (thermodynamic solubility <10 μg/mL at any pH) that we recognized as a limiting factor for absorption, led us to direct our efforts to expand this series toward more selective, stable and soluble derivatives.

Recently, in a series of similar A_{2B} antagonists, amide bond was replaced by heterocyclic 5-membered rings leading to compounds such as (**2**) (Fig. 1) in order to achieve metabolic stability and oral bioavailability.[9]

From our lead compound **1** bearing a phenoxyacetamide moiety, we synthesized a number of pyrrolopyrimidine derivatives with a metabolically stable benzenesulfonamide group.[10] Additionally, in order to increase solubility and achieve oral bioavailability we installed a basic ionizable function at the sulfonamide moiety.

Synthesis of compounds of general formula **3** (Scheme 1) was initiated by reaction of a monosubstituted urea derivative with diketene. Further alkylation followed

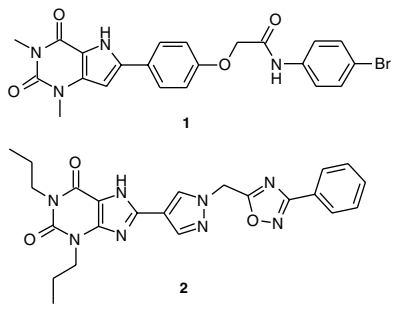
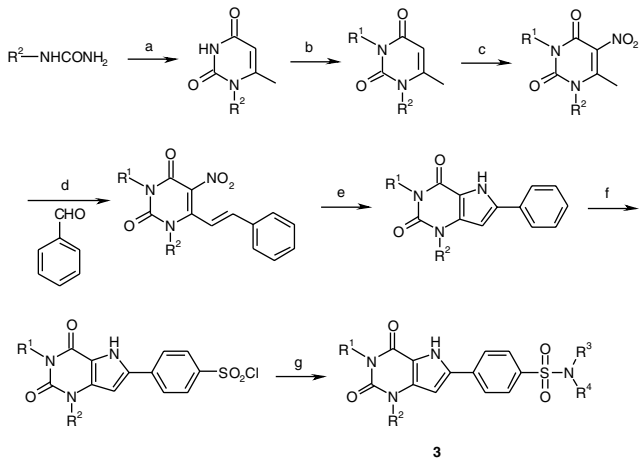


Figure 1. Structure of compounds **1** and **2**.



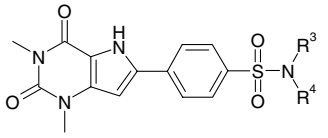
Scheme 1. General synthesis of 6-phenylpyrrolopyrimidines. Reagents and conditions: (a) diketene, AcOH, reflux (60–75%); (b) Me_2SO_4 or R^1I, 4 N NaOH, EtOH, 25 °C (88–99%); (c) HNO_3, H_2SO_4, 0 °C (81–94%); (d) PhCHO, piperidine, 3Å molecular sieves, EtOH, reflux (43–73%); (e) $Na_2S_2O_4$, formic acid, reflux (35–94%); (f) $ClSO_3H$, $SOCl_2$, 0 °C (51–92%); (g) HNR^3R^4, TEA or polymer supported morpholine, DCM, 25 °C (35–75%).

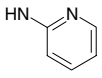
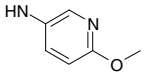
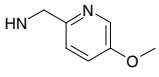
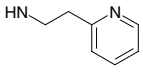
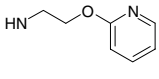
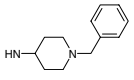
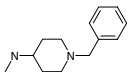
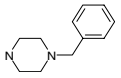
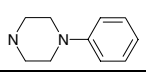
by conventional nitration gave the 6-methyl-5-nitrouracil derivatives. Condensation with benzaldehyde and subsequent reductive cyclization provided the 6-phenylpyrrolopyrimidine nucleus.

Regioselective chlorosulfonylation occurs *para* to the phenyl group in the presence of chlorosulfonic acid and thionyl chloride, to provide the sulfonyl chloride derivatives. The final step of sulfonamide formation was achieved using either triethylamine or polymer supported morpholine as a base. The latter reagent allowed the use of solution phase parallelization techniques to systematically explore SAR around the sulfonamide moiety.

All compounds described herein were tested for their ability to bind the A_{2B} adenosine receptor. Binding affinities for A_1 and A_3 receptors were determined for the interesting compounds.[11] As shown in Table 1, we first explored the variation of the sulfonamide group keeping a methyl group in R^1 and R^2. Our initial idea was testing the presence of moieties of ranging basicity such as pyridine, piperidine or piperazine. Pyridine ring either

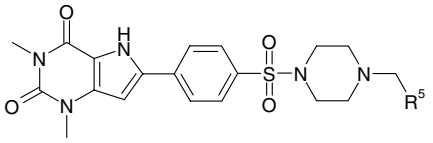
Table 1. Structure and binding affinities of selected sulfonamides bearing 1,3-dimethyl groups at the pyrrolopyrimidine core



Compound	NR^3R^4	hA_{2B} IC_{50}[a] (nM)	hA_1 IC_{50}[a] (nM)	hA_3 IC_{50}[a] (nM)
4		12	68	1354
5		18	54	1525
6		20	101	38,050
7		14	150	2085
8		17	396	6438
9		43	—	—
10		90	—	—
11		16	415	3169
12		6	—	—

[a] Values are means of two experiments.

Table 2. SAR around selected benzylpiperazinyl sulfonyl derivatives



Compound	R^5	hA_{2B} IC_{50}[a] (nM)	hA_1 IC_{50}[a] (nM)	hA_3 IC_{50}[a] (nM)
11	Ph	16	415	3169
13	3-F–Ph	7	152	8888
14	4-F–Ph	10	—	—
15	2,4-DiF–Ph	7	126	—
16	4-Br–Ph	1	183	12,260
17	5-Chloro-2-thienyl	4	102	5249
18	4-Pyridyl	27	—	—

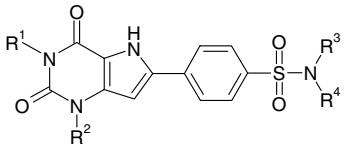
[a] Values are means of two experiments.

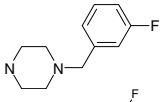
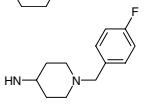
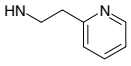
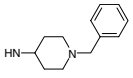
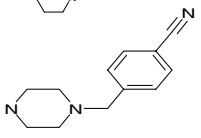
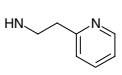
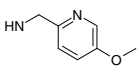
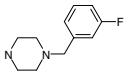
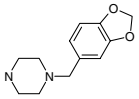
directly attached to the sulfonamide moiety (compounds **4** and **5**) or via a one, two or three atom linker (compounds **6**–**8**) showed similar affinities for the A_{2B} adenosine receptor (IC_{50} < 20 nM) and variable selectivities versus A_1 adenosine receptor. Concerning A_3 adenosine receptor, all products show a very good selectivity. Although, basic amines at the sulfonamide moiety such as those in compounds **4**–**8** showed good affinities, *N*-(1-benzylpiperidine-4-yl)sulfonamides **9**–**10** were less potent. The (4-benzylpiperazin-1-yl)sulfonyl derivative **11** came out as one of the most promising groups both in terms of potency and selectivity.

Even though compound **12** showed the highest potency, we did not further characterize this product because of the low basicity of the phenylpiperazino moiety, leading to a very limited solubility. Actually, while compound **12** showed a thermodynamic solubility lower than 10 μg/mL at any pH, compounds **7** and **11** displayed increased solubility in acidic media (pH: 1.2, 57 and 70 μg/mL, respectively).

Taking benzylpiperazines as a promising starting point we explored the SAR of diversely substituted *N*-arylmethylpiperazines. As can be seen in Table 2, substitution

Table 3. Structure and binding affinities of selected sulfonamides bearing diverse alkyl groups at positions 1 and 3 of the pyrrolopyrimidine core



Compound	R^1	R^2	NR^3R^4	hA_{2B} IC_{50}[a] (nM)	hA_1 IC_{50}[a] (nM)	hA_3 IC_{50}[a] (nM)
19	Et	Et		15	350	21,880
20	Et	Et		12	205	4314
21	Et	Et		75	140	18,950
22	*n*Pr	*n*Pr		18	59	1467
23	*n*Pr	Me		6	370	950
24	*n*Pr	Me		17	123	844
25	*n*Pr	Me		15	12	7600
26	Me	*n*Pr		17	—	—
27	Me	*n*Pr		13	102	14,860

[a] Values are means of two experiments.

with halogens at the phenyl ring of benzylpiperazines increased potency for the A_{2B} adenosine receptor up to the low nanomolar level (compounds **13**–**16**) and particularly compound **16** showed an outstanding potency (IC_{50} A_{2B}: 1 nM) and good selectivity (A_1: 183x; A_3: 12260x) relative to compound **11**. Substituted thienyl rings (compound **17**) showed a very good potency inhibiting A_{2B} adenosine receptor. In the case of substitution with a 4-pyridyl (compound **18**), a lower affinity was observed.

In order to expand SAR and further improve selectivity, we next studied the effect of variation of the R^1 and R^2 substituents at the pyrimidinedione central core by using combinations with methyl, ethyl and *n*-propyl groups with some of the best NR^3R^4 moieties identified (Table 3). Lower alkyl groups in R^1 and R^2 have proven to be the preferred substituents in this type of structures.[8–10] In compounds **19**–**27**, although potency antagonizing A_{2B} adenosine receptor is acceptable (most of them showing $IC_{50} < 20$ nM) we were not able to observe a clear improvement in selectivity versus A_1 adenosine receptor. Only in the case of compound **23** (*p*-cyanobenzylpiperazine) we observed excellent potency (IC_{50} A_{2B}: 6 nM) and a good selectivity against A_1 and A_3 adenosine receptors (A_1: 62x; A_3: 160x).

Compounds **7** and **11** were chosen as representative for pharmacokinetics. When dosed orally at 10 mg/kg in rats moderate AUC of 985 and 252 ng h/mL, respectively, with a t_{max} in both cases of 6 h. were observed. These results can be considered as an improvement since after oral administration of compound **1** (10 mg/kg) in rat, an AUC < 20 ng h/mL was observed.

In conclusion, we have identified a series of pyrrolopyrimidin-6-yl benzenesulfonamides as potent A_{2B} adenosine receptor antagonists and selective versus A_1 and A_3 adenosine receptors with improved physicochemical properties, resulting in an increased oral bioavailability.

Furthermore, compounds **16** and **23** showed an outstanding profile of potency against A_{2B} adenosine receptor (IC_{50}: 1 and 6 nM, respectively) as well as good selectivity versus A_1 and A_3.

The overall pharmacological activity of the pyrrolopyrimidine benzenesulfonamides suggests that these novel compounds constitute a promising starting point to develop novel A_{2B} antagonists for clinical progress.

References and notes

1. (a) Daly, J. W.; Padgett, W. L. *Biochem. Pharmacol.* **1992**, *43*, 1089; (b) Peakman, M. C.; Hill, S. J. *Br. J. Pharmacol.* **1994**, *111*, 191; (c) Zhou, Q. Y.; Li, C.; Olah, M. E.; Johnson, R. A.; Stiles, G. L.; Civelli, O. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7432.
2. Hasko, G.; Cronstein, B. N. *Trends Immunol.* **2004**, *21*, 33.
3. Fozard, J. R. *Curr. Opin. Pharmacol.* **2003**, *3*, 264.
4. Driver, A. G.; Kukoly, C. A.; Ali, S.; Mustafa, S. J. *Am. Rev. Respir. Dis.* **1993**, *148*, 91.
5. (a) Meade, C. J.; Worrall, L.; Hayes, D.; Protin, U. *Biochem. Pharmacol.* **2001**, *64*, 317; (b) Blackburn, M. R.; Lee, C. G.; Young, H. W.; Zhu, Z.; Chunn, J. L.; Kang, M. J.; Banerjee, S. K.; Elias, J. A. *J. Clin. Invest.* **2003**, *112*, 332; (c) Feoktistov, I.; Goldstein, A.; Sheller, J. R.; Schwartz, L. B.; Biaggioni, I. *Drug Dev. Res.* **2003**, *58*, 461; (d) Ryzhov, S.; Goldstein, A. E.; Matafonov, A.; Zeng, D.; Biaggioni, I.; Feoktistov, I. *J. Immunol.* **2004**, *72*, 7726; (e) Zhong, H.; Belardinelli, L.; Maa, T.; Feoktistov, I.; Biaggioni, I.; Zeng, D. *Am. J. Respir. Cell Mol. Biol.* **2004**, *301*, 118; (f) Zhong, H.; Belardinelli, L.; Maa, T.; Zeng, D. *Am. J. Respir. Cell Mol. Biol.* **2005**, *32*, 2.
6. Barnes, P. J. *Am. J. Respir. Crit. Care Med.* **2003**, *167*, 813.
7. Holgate, S. T. *Br. J. Pharmacol.* **2005**, *145*, 1009.
8. (a) Carotti, A.; Cadavid, M. I.; Centeno, N. B.; Esteve, C.; Loza, M. I.; Martinez, A.; Nieto, R.; Raviña, E.; Sanz, F.; Segarra, V.; Sotelo, E.; Stefanachi, A.; Vidal, B. *J. Med. Chem.* **2006**, *49*, 282; (b) Vidal, B.; Esteve, C.; Segarra, V.; Raviña, E.; Fernandez, F.; Loza, M. I.; Sanz, F. PCT Int. Appl. WO 03000694, 2003.
9. (a) Zablocki, J.; Kalla, R.; Perry, T.; Palle, V.; Varkhedkar, V.; Xiao, D.; Piscopio, A.; Maa, T.; Gimbel, A.; Hao, J.; Chu, N.; Leung, K.; Zeng, D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 609; (b) Elzein, E.; Kalla, R.; Li, X.; Perry, T.; Parkhill, E.; Palle, V.; Varkhedkar, V.; Gimbel, A.; Zeng, D.; Lustig, D.; Leung, K.; Zablocki, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 302.
10. Vidal, B.; Esteve, C. PCT Int. Appl. WO 03082873, 2003.
11. Biological assay procedures. (i) A_1 receptor assay: a radioligand binding assay was used with [^{3}H]DPCPX as a ligand and membranes from CHO cells transfected with human A_1 receptor; (ii) A_{2B} receptor assay: a radioligand binding assay was used with [^{3}H]DPCPX as a ligand and membranes from HEK 293 cells transfected with human A_{2B} receptor; (iii) A_3 receptor assay: a radioligand binding assay was used with [^{3}H]NECA as a ligand and membranes from HeLa cells transfected with human A_3 receptor.