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Antagonists of the human A_{2A} receptor. Part 5: Highly bio-available pyrimidine-4-carboxamides

Roger J. Gillespie [a], Samantha J. Bamford [a], Suneel Gaur [a], Allan M. Jordan [b,*], Joanne Lerpiniere [a], Howard L. Mansell [a], Gemma C. Stratton [a]

[a] Vernalis (R+D) Ltd., 613 Reading Road, Winnersh Wokingham, RG41 5UA, UK
[b] Vernalis (R+D) Ltd., Granta Park, Cambridge, CB21 6GB, UK

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ABSTRACT

A novel series of antagonists of the human A_{2A} receptor have been identified and have been shown to display good potency and high degrees of selectivity over other receptor sub-types. Displaying in vivo potency in commonly used disease models and high oral bio-availability, this class of compounds may serve as clinically useful treatments for the relief of the symptoms associated with Parkinson's disease.
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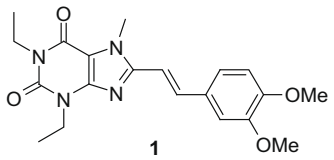
The human adenosine receptor is a G-protein coupled receptor which is delineated into four sub-types, namely the A_1, A_{2A}, A_{2B} and A_3 receptors. The A_{2A} receptor is highly expressed in the striatum and is involved in smooth, well co-ordinated muscle movement.[1] Recent evidence has highlighted this receptor as a point of intervention for the treatment of symptoms associated with Parkinson's disease.[2] In this debilitating condition, striatal dopaminergic neurons are degraded, reducing dopamine levels and resulting in an imbalance of dopaminergic and GABAergic neurotransmitter signaling.[3] Although this reduction can be combated by administration of the dopamine precursor L-DOPA, this treatment is far from satisfactory and causes considerable side-effects.[4] As an alternative therapeutic approach, antagonism of the A_{2A} receptor reduces adenosine signaling and restores balance to the signaling pathway, re-introducing control over muscle movement.[5]



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Recently, there has been much progress made in the discovery of small molecules as A_{2A} antagonists and compounds such as KW-6002 **1** have been the subject of clinical evaluation. This xanthine-based compound has been reported to have efficacy in models of the disease[6] without inducing hyperactivity or inducing dyskinesias.[7] More recently, the compound has been the subject of clinical evaluation,[8,9] but failed to meet primary endpoints in two of the three pivotal trials.[10] This outcome may be attributable, at least in part, to metabolic issues associated with the compound.[11]Additional non-xanthine compounds such as ZM241385 and SCH58261 have been reported and extensively studied.[12,13] However, despite the recent publication of the crystal structure of the human A_{2A} receptor and its potential utility in structure guided drug design,[14] the discovery of selective, potent and metabolically stable compounds with good oral bioavailability remains a challenging proposition.[15] In an effort to address this need, we have recently reported how investigations of the anti-malarial compound mefloquine **2**[16] led to the discovery of V2006/BIIB-014 **3**, a novel A_{2A} antagonist now in clinical trials for the relief of symptoms associated with Parkinson's disease.[17]

Herein, we report our further research in this area and reveal a novel scaffold which represents one of the simplest classes of adenosine A_{2A} antagonists.[18] Furthermore, these derivatives possess high affinity, selectivity over other adenosine receptor subtypes and a promising in vivo profile, both in terms of pharmacokinetics and activity in common disease models.

* Corresponding author. Tel.: +44 0 1223 895 555; fax +44 0 1223 895 556.
E-mail address: a.jordan@vernalis.com (A.M. Jordan).

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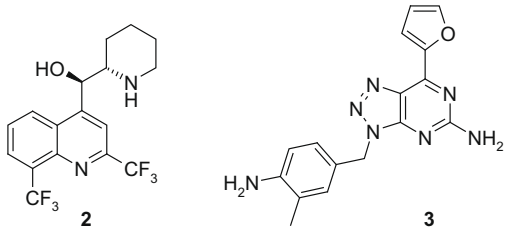
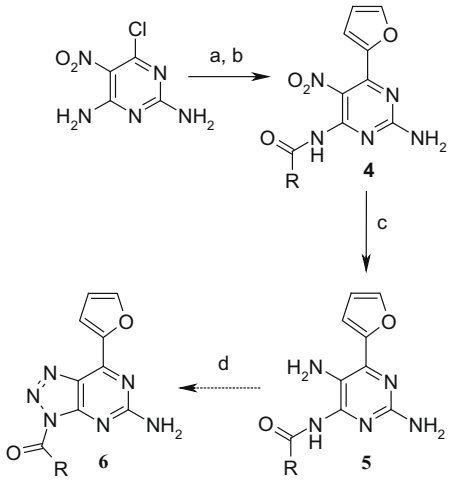


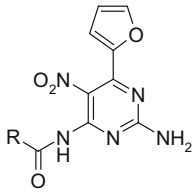
Our work in this area began with the triazolopyrimidine series, typified by **3**. We proposed that the synthesis of a series of related analogues could be achieved via a 5-nitropyrimidine precursor **4**, which could be reduced to the 2,5-diaminopyrimidine **5**. We then hoped this derivative could be acylated and cyclised to give acyl triazolopyrimidine derivatives such as **6** (Scheme 1), though this methodology proved somewhat troublesome.

During these investigations, we were surprised to find that the 2-amino-5-nitro pyrimidine precursors typified by **7** and **8** possessed moderate activity against the A_{2A} receptor (Table 1).[19] Moreover, these highly compact and efficient derivatives (ligand efficiency 0.37–0.39) were also found to demonstrate measurable activity in the haloperidol-induced hypolocomotion assay (a commonly used model for Parkinson's disease) after oral dosing at 50 mg kg^{-1}.



Scheme 1. Reagents and conditions: (a) furan-2-boronic acid, $Pd(PPh_3)_4$, $NaHCO_3$, THF, reflux, 18 h, 49%; (b) ArCOCl, pyridine, 80 °C, 16 h, 6–82%; (c) H_2, 10% Pd/C, EtOH/EtOAc, 3 h, quant; (d) $NaNO_2$, HCl, EtOH, 0 °C, 1 h, then rt, 1 h.

Table 1
Receptor binding affinities for selected nitropyrimidines[18]



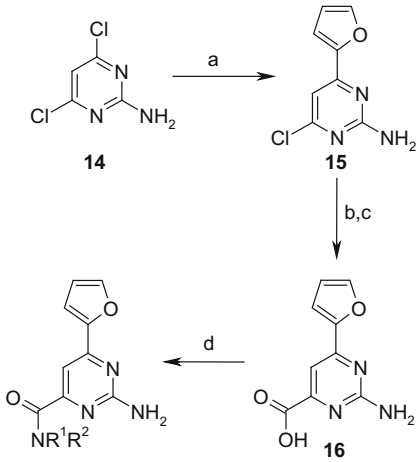
Compound	R	A_{2A} K_i (nM)	A_1 K_i (nM)
7	Phenyl	308	2062
8	4-Pyridyl	153	2004
9	2-F–Ph	23	311
10	2-MeO–Ph	170	770
11	2-CF_3O–Ph	16	298
12	3-Pyridyl	220	3540
13	2-MeO–$PhCH_2$	10	884

We hypothesised that the π-electrons of the aromatic nitro group were adopting a 'pseudo-ring' binding orientation which was co-planar with the pyrimidine ring,[20] mimicking the 'A' ring of the triazolopyrimidine scaffold and retaining much of the binding affinity previously observed in that series. Though the observed binding affinities were interesting, it was clear that further improvements in selectivity and oral activity were required. Initial efforts focused upon the optimization of the amide substituent. A small number of *ortho*-substituted benzoic acids were employed and nicotinic acid derivatives were also investigated, to aid solubility. Representative data for this series are described in Table 1. More impressive gains were obtained using phenylacetic acid derivatives such as **13**, where homologation gave an appreciable increase in potency and considerable gains in selectivity over the A_1 receptor, compared to the corresponding benzoate **10**.

We then reasoned that, if the nitro moiety was indeed mimicking the π-electrons of the triazolopyrimidine 'A' ring as we suspected, we could additionally emulate this electron distribution by reversing the amide at C-4 and deleting the nitro group at C-5. If effective, this simple change would both simplify the chemistry strategy and allow rapid exploitation of this series. Moreover, if the π-electrons of the nitro moiety were indeed crucial for activity, we predicted metabolic reduction to the corresponding amine would eradicate in vivo activity. Removal of this liability at an early stage was therefore highly appealing.

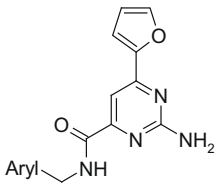
To access the desired compounds, the required furyl pyrimidine acid was prepared from the commercial 2-amino-4,6-dichloropyrimidine **14** (Scheme 2). A Stille coupling selectively yielded the mono-arylated product **15**. Displacement of the chlorine at C-4 with sodium cyanide, followed by acidic hydrolysis gave the key acid intermediate **16**, which could be elaborated with a variety of methylamine substituents using solid-supported carbodiimide resin as the coupling reagent. Drawing upon the observations made in the previous series of phenylacetic acid derivatives, benzylamines were employed in the first round of syntheses to give side-chains of similar dimensions.

Immediate improvements in the overall profiles of the compounds were seen compared to the respective nitropyrimidines (e.g., compounds **18** and **9**, respectively). Binding affinity against the A_{2A} receptor had improved almost sevenfold (yielding a concommitant increase in ligand efficiency from 0.37 to an average



Scheme 2. Reagents and conditions: (a) 2-(tributylstannyl)furan, $PdCl_2(PPh_3)_2$, DMF, 80 °C, 18 h, 57%; (b) NaCN, DABCO, DMSO, 6 days, rt, 77%; (c) H_2SO_4, rt, 2 h, 90%; (d) R^1R^2NH, PS-CDI, HOBt, DMF, rt, 24 h, 26–85%.

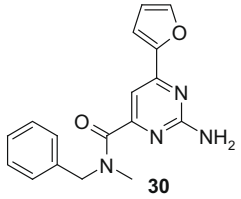
Table 2
Receptor binding affinities for pyrimidine-4-carboxamides[18]



Compound	Aryl	A_{2A} K_i (nM)	A_1 K_i (nM)
17	Ph	7.6	58
18	2-F–Ph	3.5	54
19	3-F–Ph	4.8	39
20	4-F–Ph	20	140
21	2-Cl–Ph	2.6	72
22	3-Cl–Ph	3.7	12.2
23	4-Cl–Ph	17.5	80
24	2-Me–Ph	2.2	73
25	3-Me–Ph	2.6	38
26	4-Me–Ph	9.5	102
27	2-MeO–Ph	3.5	34.5
28	3-MeO–Ph	6.1	47
29	4-MeO–Ph	79	157

of 0.47 for these initial derivatives) whilst still maintaining 15-fold selectivity against the A_1 receptor. Selected results from these studies are detailed in Table 2.

This data indicated that *ortho*-substituents were favoured in this system, giving potency gains over the corresponding *meta*-substituents and, more dramatically, *para*-substituents. Though *ortho*- and *meta*-substitution tended to give similar selectivity profiles, with *ortho*-substitution being slightly more beneficial, those compounds bearing *para*-substituents tended to demonstrate poor selectivity against the A_1 receptor. The difference in activity between the 4-methyl and 4-methoxy derivatives **26** and **29** perhaps indicates that the *para*-substituents are directed into a sterically encumbered pocket, with little room to accommodate larger moieties. Next, we investigated N-alkylation, preparing compound **30**, the *N*-methyl analogue of **17**. This compound exhibited a dramatic loss in binding affinity, with K_is of 552 nM against the A_{2A} receptor and over 3500 nM against the A_1 receptor. This indicated a clear preference for a secondary, rather than tertiary amide, at this position.



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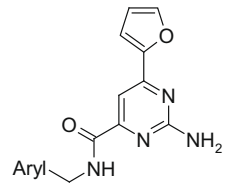
Furthermore, initial data suggested that heteroatoms at the 2- or 3-position of the aromatic ring gave good activity and reasonable selectivity, whereas more removed heteroatoms give a poorer profile, perhaps suggesting a more non-polar environment in this region (Table 3). As soluble, bio-available A_{2A} antagonists still remain a challenging problem, a selection of pyridyl compounds were also prepared, which displayed a useful three- to fivefold enhancement in aqueous solubility.[21]

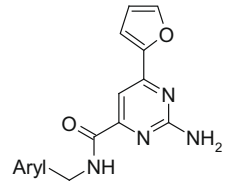
Further investigations were then undertaken with the more promising compounds from these studies, to evaluate their potential as lead compounds toward treatments for Parkinson's disease. Firstly, the functional activity of these compounds was determined by assessing Ca^{2+} mobilization via a Fluorescence Imaging Plate Reader (FLIPR) assay.[22] All compounds tested were found to be functional antagonists of the A_{2A} receptor, failed to affect the maximal response of a known agonist when dosed in tandem and exhibited no appreciable agonist activity ($EC_{50} \gg 10\ \mu M$). Compounds were then evaluated for their specificity against the other two adenosine receptor subtypes (Table 4).

Table 3
Receptor binding affinities for heterocyclic pyrimidine-4-carboxamide derivatives[18]



Compound	Aryl	A_{2A} K_i (nM)	A_1 K_i (nM)
31	2-Pyridyl	4.3	122
32	3-Pyridyl	9.4	124
33	4-Pyridyl	388	1342
34	2-Furyl	14	214
35	(6′-Amino)-pyridyl	7	131
36	(6′-Hydroxymethyl)-2-pyridyl	9.3	260
37	(6′-Methoxymethyl)-2-pyridyl	1.7	42

Table 4
Receptor subtype selectivity for selected compounds[18]



Compound	Aryl	A_{2A} K_i (nM)	A_{2B} K_i (nM)	A_3 K_i (nM)
17	Ph	7.6	858	857
18	2-F–Ph	3.5	67	335
19	3-F–Ph	4.8	924	1063
21	2-Cl–Ph	2.6	137	414
22	3-Cl–Ph	3.7	184	507
24	2-Me–Ph	2.2	917	586
25	3-Me–Ph	2.6	402	1526
26	4-Me–Ph	9.5	1240	1848
27	2-MeO–Ph	3.5	156	402
28	3-MeO–Ph	6.1	121	1102
31	2-Pyridyl	4.3	754	1390
32	3-Pyridyl	9.4	5875	6437
35	6′-NH_2-2-Pyr	7	444	1483
37	6′-($MeOCH_2$)Pyr	1.7	460	1740

Activity against the A_{2A} receptor was generally observed to be between 50 and 200-fold greater than that observed against the A_{2B} and A_3 receptors, with selectively against A_3 generally greater than that observed against A_{2B}. However, compounds such as **25**, **32** and **37** demonstrated selectivity of up to 1000-fold in some cases.

Carboxamide derivatives displaying a binding affinity of less than 10 nM at the A_{2A} receptor and greater than 15-fold selectivity against the A_1 receptor were then investigated for their ability to reduce Parkinson's-like symptoms in the HaloLMA assay, a validated in vivo model of the disease.[23] In this model, a temporary form of Parkinson's disease can be induced by using agents which block dopaminergic neurotransmission, such as the dopamine receptor antagonist haloperidol. Animals treated in this manner show an impairment of movement, which mirrors that observed in Parkinson's disease. We assessed the in vivo A_{2A} antagonist properties of novel compounds by monitoring reversal of the

Table 5
Minimum oral dose required for reversal of haloperidol-induced hypolocomotion in mice[23,24]

Compound	Minimum effective dose (mg kg^{-1})
18	30
21	30
24	30
25	3
27	20
31	3
35	1
36	30
37	0.1

reduction in locomotor activity induced by haloperidol in mice. Data for this assay are summarised in Table 5.

Derivatives **18**, **21**, **24** and **36** were found to have some activity, partially reversing haloperidol-induced hypolocomotion in mice when dosed orally at 30 mg kg^{-1} whilst compound **27** fared slightly better, showing activity at 20 mg kg^{-1}. However, the pyridyl compound **31**, alongside the *meta*-methyl substituted benzylamine derivative **25**, showed considerably better in vivo activity, demonstrating reversal of hypolocomotion when dosed orally at just 3 mg kg^{-1}. Furthermore, derivative **35**, which displayed enhanced solubility, demonstrated activity at 1 mg kg^{-1} in the same protocol. Though these activities were encouraging, we were very pleased to observe that **37**, our most potent compound, retained activity in vivo, reversing haloperidol-induced hypolocomotion at just 0.1 mg kg^{-1} when dosed orally. This data prompted us to assess the physiochemical properties of **37** in further detail.

As we had anticipated, solubility was found to be very good, demonstrating a maximal aqueous solubility of over 450 μM. Cytochrome P450 inhibition was also investigated and shown to be negligible for a variety of P450 isoforms. Initial pharmacokinetic studies in rats revealed that the compound exhibited an oral plasma half-life of approximately 1 h and showed a remarkable oral bio-availability of around 90%. Furthermore, brain exposure was found to be excellent with 85% uptake,[25] suggesting our molecular profile was ideal for penetration through the blood-brain barrier. Once in the brain, the compound again demonstrated a half-life of around 1 h, which when coupled with a plasma T_{max} of 15–30 min would suggest the compound is likely to have a rapid onset of action post-dose. These properties undoubtedly contribute to the potent activity seen in the HaloLMA model, where locomotor activity is assessed over 1.25 h post-dose period.

These data compare very favourably with the aforementioned KW-6002 **1**. In our hands, **1** exhibits a K_i of 36 nM against the human A_{2A} receptor and is 80-fold, 50-fold and >80-fold selective against the human A_1, A_{2B} and A_3 receptors, respectively. In the HaloLMA assay, compound **1** exhibits reversal of hypolocomotion in mice with a minimum effective oral dose of 0.3 mg kg^{-1}.

In conclusion, we have revealed a novel, small-molecule class of adenosine A_{2A} receptor antagonists which display high degrees of potency, reasonable selectivity against the other receptor subtypes and oral activity in an in vivo model of Parkinson's disease. Though the compounds described herein display slightly reduced selectivity against the A_1 receptor, compared to the clinically investigated KW-6002 **1**, in many cases selectivity against the A_{2B} and A_3 receptors is increased. Furthermore, potency against the A_{2A} receptor is generally equal or better and our compounds display improved in vivo activity than that observed for **1**. Initial pharmacokinetic analysis indicates good solubility and oral bio-availability, excellent brain penetration and rapid onset of action. Further optimization of this series, based upon these preliminary findings, will be elaborated upon in later reports.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.142.

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24. 1.5 hours prior to testing, mice were administered with haloperidol (0.2 mg kg^{-1} sc). Test compounds were orally administered 15 min prior to testing. Horizontal locomotor activity (as measured by beam breaks) was assessed over 1 h.
25. The measured brain uptake was determined in male Sprague-Dawley rats from the brain/plasma AUC_{last} ratio, following intravenous administration of **37** at 2 mg kg^{-1}.