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Structure–Activity Studies of 5-Substituted Pyridopyrimidines as Adenosine Kinase Inhibitors

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Abstract—The synthesis and SAR of a novel series of non-nucleoside pyridopyrimidine inhibitors of the enzyme adenosine kinase (AK) are described. It was found that pyridopyrimidines with a broad range of medium and large non-polar substituents at the 5-position potently inhibited AK activity. A narrower range of analogues was capable of potently inhibiting adenosine phosphorylation in intact cells indicating an enhanced ability of these analogues to penetrate cell membranes. Potent AK inhibitors were found to effectively reduce nociception in animal models of thermal hyperalgesia and persistent pain.

Introduction

Endogenously produced adenosine (ADO) serves a number of roles in the body, but it is especially important as an extracellular messenger where it acts at specific receptors on the cell surface to modulate neuronal activity and inflammation.[1] In spite of extensive effort, the direct pharmacological modulation of adenosine receptors with agonists has not yielded useful drug candidates for human use, due to the prevalence of mechanism based side effects (prominently hemodynamic effects). A rationale for a therapeutic approach targeting an *indirect* modulation of ADO receptors has been proposed as providing 'site and event selectivity', with an enhanced therapeutic window.[2] Damaged tissues produce elevated levels of ADO, and inhibition of the metabolism of endogenously produced ADO may selectively amplify its local action in comparison with undesired systemic actions.[3] AK plays a major regulatory role in metabolizing and inactivating ADO by rephosphorylation to AMP. ADO and ADO receptor agonists have shown analgesic actions in both clinical and preclinical models.[3] Our interest has been to prepare AK inhibitors for use as a novel class of analgesics, free of the side effects of more traditional analgesics such as opiates.

The substrate ADO **1** is highly polar and rapidly metabolized, but has nonetheless been used as the starting point for the design of AK inhibitors through rational drug design (Scheme 1).[4] However, we have more recently described a novel series of pyridopyrimidine AK inhibitors **2** developed by optimization of the high throughput screening hit **3** (IC_{50} 400 nM).[5] We anticipated improved membrane penetration and greater metabolic stability for compounds based on **2**, since these are more lipophilic and lack the hydroxyl groups seen in nucleoside-like inhibitors. In this report, we describe large gains in potency upon optimization of the R^5 position in **2**, in which the best compounds exhibited up to a 2000-fold boost in potency, in comparison with the unsubstituted analogues.

Chemistry

The synthesis of 5-aryl pyridopyrimidines[6] was carried out as has been described, where aryl aldehydes **4** were condensed with aryl ketones **5** in the presence of NH_4OAc in ethanol or benzene to produce the amino cyano pyridine intermediates **6** ($R^5 = Ar$, Scheme 2). For compounds **6**,

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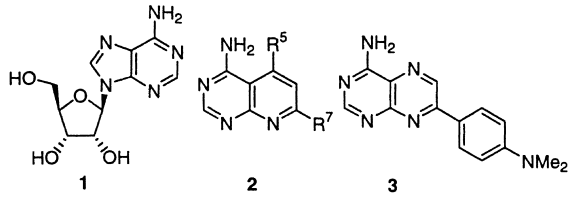


Scheme 1. Structures of substrate adenosine **1**, pyridopyrimdine AK inhibitors **2**, and screening lead structure **3**.

these were subsequently cyclized by heating in formamide to give target **2**.

However these conditions failed on two counts when attempting to prepare compounds **2** containing an alkyl group at R^5. Firstly, under the standard literature conditions, heating aliphatic aldehydes bearing an α-proton with NH_4OAc in ethanol or benzene gave only aldol-derived self condensation products. However, it was found that aliphatic aldehydes **4** could be *precondensed* with malononitrile to produce dicyanoethylenes **7**, which after isolation, were then condensed with ketones **5** and NH_4OAc to give high yields of **6**. It was found that 1,2-dichloroethane was a much superior (and in some cases essential) solvent to benzene or alcohol for this condensation.

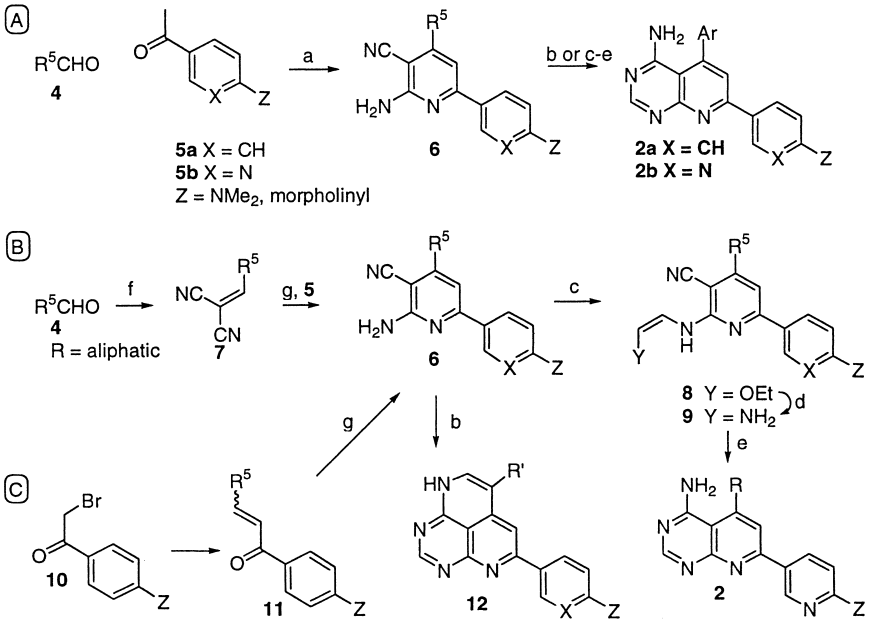
The conversion of **6** (where R^5 = alkyl) to **2** under the standard conditions reported in the literature (heating with formamide or formamidine) was not generally successful, owing to the unexpected formation of the novel tricyclic byproducts **12**. A more efficient procedure for this transformation was a three step process where **6** was heated with $HC(OEt)_3$ to produce an iminoether **8**, which was then stirred with NH_3 to give an amidine **9**, which was then thermally cyclized to produce **2**. This process was not only successful[7] in eliminating the formation of the side product **12** for compounds where R^5 was alkyl, but was found to produce cleaner products and higher yields than the standard methodology for all compounds **2**.

An alternative method for the formation of **2** where R^5 = alkyl was found to be effective where X = CH. In this case, **10** was converted first to an ylide by reaction with Ph_3P, then treatment with NaOH, followed by reaction with aliphatic aldehydes **4** to produce the chalcone **11**. Cyclization with $CH_2(CN)_2$ gave **6**, which was converted to the target **2** as described above.

Biological Results and Discussion

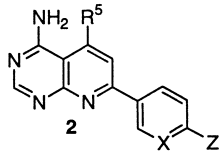
The importance of the R^5 group in modulating cytosolic AK inhibition can be readily seen in Table 1.[8] The trend was for AK inhibitory potency to rapidly increase as the size of the substituent at R^5 was increased from H (**13** = 733 nM and **27** = 562 nM) to methyl (**14** = 47 nM), to cyclopropyl (**15** = 55 nM), to butyl (**16** = 11 nM), to phenyl (**18** = 7 nM), to cyclohexyl (**17** = 8 nM, **39** = 3 nM) and 2-bromobenzyl (**43** = 0.17 nM). Variation of the substitution of the aryl groups led generally to good potency, with a trend for the compounds with more polar groups to have reduced activity (compare **15**–**22** vs **23**–**26** and **40** vs **39**). This pattern of activity suggests that the AK possesses a binding pocket that prefers pyridopyrimidines of structure **2** to have medium to large R^5 substituents with lipophilic properties. When these conditions are met, compounds were found to consistently possess potency in the low nanomolar range.

In order for a compound to display in vivo activity in pain models (Table 2), potent inhibitory activity at cytosolic AK is necessary. However, a very important



Scheme 2. Reagents: (a) $CH_2(CN)_2$, NH_4OAc, benzene, 80 °C; (b) $HCONH_2$ 170 °C; (c) $HC(OEt)_3$, PTSA, 140 °C; (d) NH_3; (e) 140 °C, 1,2-dichlorobenzene; (f) MgO, $CH_2(CN)_2$, CH_2Cl_2; (g) $CH_2(CN)_2$, NH_4OAc, CH_2ClCH_2Cl, 80 °C; (h) (i) Ph_3P, Et_3N, CH_3CN; (ii) NaOH; (iii) R^5CHO, $PhCH_3$; (i) (Z = Cl → Z = NR''_2) HNR''_2, DMSO, 100 °C.

Table 1. In vitro inhibition of AK in cytosolic and intact assays[b]



Compound	X	Z	R^5 substituent	Cytosolic AK[a] inhibition IC_{50} (nM)		Intact cell AK[a] inhibition IC_{50} (nM)	
				Mean	SEM	Mean	SEM
13	CH	$N(CH_3)_2$	H	733	58	>10,000	
14	CH	$N(CH_3)_2$	CH_3	47	30	>1000	
15	CH	$N(CH_3)_2$	Cyclopropyl	55	13	825	150
16	CH	$N(CH_3)_2$	Butyl	11	10	550	260
17	CH	$N(CH_3)_2$	Cyclohexyl	8	1	470	230
18	CH	$N(CH_3)_2$	Ph	7	2	470	58
19	CH	$N(CH_3)_2$	Ph(3-Cl)	15	10	78	18
20	CH	$N(CH_3)_2$	Ph(3-Br)	5	2	170	58
21	CH	$N(CH_3)_2$	Ph(3-I)	17	14	120	30
22	CH	$N(CH_3)_2$	Ph(3-CF_3)	36	25	225	35
23	CH	$N(CH_3)_2$	Ph(3-OCH_3)	85	78	400	140
24	CH	$N(CH_3)_2$	Ph(3-OH)	44	28	353	350
25	CH	$N(CH_3)_2$	Ph(3-$CONH_2$)	77	28	850	210
26	CH	$N(CH_3)_2$	Ph(3-CN)	35	26	325	35
27	N	Morpholine	H	562	280	>1000	
28	N	Morpholine	Ph(2-Br)	3	1	675	100
29	N	Morpholine	Ph(3-Br)	2	0.5	50	8
30	N	Morpholine	Ph(4-Br)	30	10	430	115
31	N	Morpholine	Ph(3-Cl)	9	2	96	7
32	N	Morpholine	Pyridin-3-yl	62	16	315	112
33	N	Morpholine	Thiophen-3-yl	13	10	550	210
34	N	Morpholine	Furan-2-yl	10	1	488	350
35	N	Morpholine	Ph(2,3-dichloro)	5	1	130	3
36	N	Morpholine	Ph(2,5-dichloro)	38	18	378	94
37	N	Morpholine	Pentan-3-yl	9	2	115	30
38	N	Morpholine	Cyclopentyl	2	1	80	16
39	N	Morpholine	Cyclohexyl	3	1	57	4
40	N	Morpholine	Tetrahydro pyran-4-yl	17	6	800	160
41	N	Morpholine	Cyclohexyl *cis* (3,5-dimethyl)	0.23	0.07	102	14
42	N	Morpholine	$C(CH_3)_2CH{=}CH$	21	5	304	90
43	N	Morpholine	CH_2Ph(2-Br)	0.17	0.02	33	20
44	N	Morpholine	$CH(CH_3)$Ph(2-Br) (racemic)	0.25	0.18	30	18

[a]Inhibition of AK and ADO phosphorylation assays were carried out as described by Jarvis et al.[7]
[b]Values represent means ± SEM determined from multiple (≥3) determinations.

additional property should also include the ability to cross cell membranes, since AK is an *intracellular* target.

In both these respects, compounds with a 7-(morpholino)pyridyl group at R^7 (X = N, **27**–**44**) generally possessed improved properties, as well as efficacy in animal pain models,[8] when compared with compounds with a 7-phenyl group at R^7 (X = CH, **13**–**26**). Analogues (**13**–**26**) with a 7-phenyl group at R^7 (X = CH) were often highly insoluble in aqueous media, whereas **27**–**44** were more soluble, and especially so when formulated as hydrochloride salts. As reflected in the AK intact cell potencies, the latter class of compounds with R^7 = 7-(morpholino)pyridyl group also contained the analogues which were most potent in the ability to penetrate intact cells and inhibit the AK enzyme (**29**, **43**, and **44**). As was expected, since activity in the intact cell AK assay was anticipated to better predict in vivo activity, members of this class were also more generally efficacious in animal pain models. Among **13**–**26** (and among >100 related analogues) only **20** displayed any in vivo activity in the rat thermal hyperalgesia assay, a model of inflammatory pain.[9] However, **20** was not active in pain models following oral administration. In contrast, among **27**–**44** several compounds were active in both the thermal hyperalgesia model and in the formalin test[10] (Table 2). Compounds with more potent AK cellular inhibition activity tended to exhibit better analgesic activity. Some analogues (especially **29**) also retained high activity following oral administration. Another reason for the generally enhanced in vivo and whole cell AK activity seen in the 7-pyridyl class of analogues may be in part due to their observed higher solubility than the 7-phenyl analogues, thereby allowing them to reach higher concentrations more quickly in cells. These potent analogues have been used as a starting point for the design of further modified analogues optimized for potent oral analgesic activity, and for selectivity versus the side effects that some analogues showed in reducing spontaneous locomotor activity.[11]

Table 2. Activity of selected compounds in in vivo (rat) pain models

Compound	Rat formalin nociception[a]	Rat carrageenan hyperalgesia[a]
	ED_{50}[b] (μmol/kg)	ED_{50}[b] (μmol/kg)
20	0.7	30
28	10	30
29	25 (60)	0.6 (5)
30	30	n.t.
31	10	20
32	n.t.[c]	>30
35	n.t.	5
37	n.t.	30
38	n.t.	10[d]
39	20	10 (5)
40	30	2 (>30)
41	>30	30
43	30	3
44	10 (100)	10

[a]Values represent compounds administered by ip injection, except for values in parentheses, which represent compounds administered orally.
[b]ED_{50}s reported for compounds for which a complete dose–response relationship was obtained with at least six animals at each dose, $p < 0.05$.
[c]Not tested.
[d]Compound administered by subcutaneous injection.

In summary, the study of the SAR of a series of 5-substituted pyridopyrimidines has led to the discovery of a family of highly potent AK inhibitors with in vivo activity.

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