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Synthesis and biological evaluation of 6,7-disubstituted 4-aminopyrido[2,3-*d*]pyrimidines as adenosine kinase inhibitors

Richard J. Perner,* Chih-Hung Lee, Meiqun Jiang, Yu-Gui Gu, Stanley DiDomenico, Erol K. Bayburt, Karen M. Alexander, Kathy L. Kohlhaas, Michael F. Jarvis, Elizabeth L. Kowaluk and Shripad S. Bhagwat†

Neuroscience Research, Global Pharmaceutical Research and Development, Abbott Laboratories, 100 Abbott Park Road, Abbott Park, IL 60064-6115, United States

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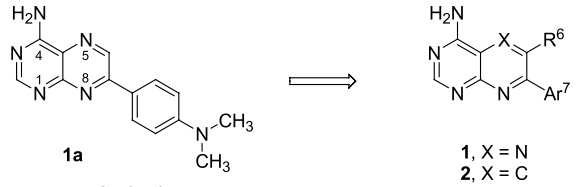
Abstract—The synthesis and structure–activity relationship of a series of 6,7-disubstituted 4-aminopyrido[2,3-*d*]pyrimidines as novel non-nucleoside adenosine kinase inhibitors is described. A variety of substituents, primarily aryl, at the C6 and C7 positions of the pyridopyrimidine core were found to yield analogues that are potent inhibitors of adenosine kinase. In contrast to the 5,7-disubstituted and 5,6,7-trisubstituted pyridopyrimidine series, these analogues exhibited only modest potency to inhibit AK in intact cells.

Adenosine kinase (AK) is a ubiquitous intracellular enzyme, which catalyzes the phosphorylation of adenosine (ADO) to adenosine monophosphate, and therefore is a key enzyme in the control of cellular concentrations of ADO. Adenosine has been characterized as a homeostatic modulator of cellular activity[1] and, as such, has several important physiological effects in the central nervous system, functioning as an endogenous anti-convulsant,[2,3] and a neuroprotective agent.[4] Adenosine has also been implicated in modulating transmission in pain pathways in the spinal cord.[5] During periods of excessive cellular activity or tissue trauma, extracellular ADO release is enhanced, which activates specific P1 purinergic receptors (A_1, A_{2A}, A_{2B}, and A_3) to elicit a variety of responses which tend to decrease cellular activity, and restore cellular function toward normal.[6,7] Since ADO has a half-life measured in seconds[8] in extracellular fluids its endogenous actions are highly localized. Therefore, selective inhibition of AK represents an attractive approach to enhance the release of endogenous ADO to the extracellular space thus benefiting from its neuroprotective, anti-convulsant, and anti-nociceptive effects.

We have pursued pyridopyrimidine derivatives as a novel class of AK inhibitors.[9–14] These compounds were derived from the pteridine compound **1a** (Fig. 1), which was identified by high-throughput screening of the Abbott compound library as a novel, non-nucleoside AK inhibitor with low micromolar potency ($AK_{(enzyme)}$: IC_{50} = 0.4 μM). The compound was inactive at other receptor, enzyme, and kinase screens, suggesting selectivity for AK.[15] Although **1a** was identified as an AK inhibitor with modest potency, it was considerably weaker at inhibiting ADO phosphorylation in intact cells ($AK_{(intact\ cell)}$: 3.6 μM) indicating poor ability to



IC_{50}(nM)	
$AK_{(enzyme)}$	440 ± 29
$AK_{(intact\ cell)}$	3600 ± 740

Figure 1. Adenosine kinase inhibitor high-throughput screening hit **1a**, and novel pyridopyrimidine AK inhibitor **2**.

Keywords: Adenosine kinase; Pyridopyrimidine.
* Corresponding author. Tel.: +1 8479371023; fax: +1 8479379195; e-mail: richard.j.perner@abbott.com
† Present address: Celgene, Signal Research Division, 5555 Oberlin Dr. San Diego, CA 92130.

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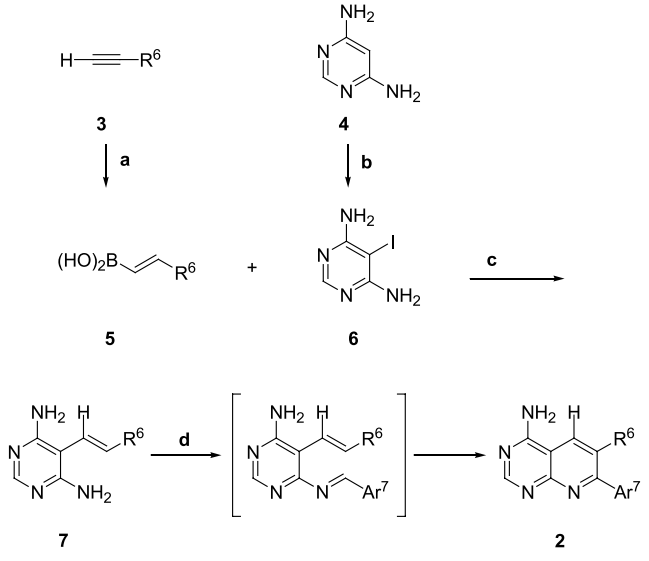
penetrate the cell membrane. Medicinal chemistry efforts directed at optimizing the potency and improving the membrane permeability of these AK inhibitors was initiated. Herein, we report our investigation on the development of 6,7-disubstituted 4-aminopyrido[2,3-*d*]pyrimidines **2** as inhibitors of adenosine kinase.

The synthesis of 6,7-disubstituted 4-aminopyrido[2,3-*d*]-pyrimidines was accomplished using the route shown in Scheme 1. An appropriately substituted acetylene **3** was treated with catecholborane followed by Suzuki coupling of the resulting boronic acid **5** with iodopyrimidine **6** to give the *trans* disubstituted alkene **7**, typically in 50–80% yield after purification. The desired products **2** were then obtained via cyclization by aza-Cope rearrangement of the intermediate formed by reaction of **7** with an aryl aldehyde at high temperature in 1,2,4-trichlorobenzene or diphenyl ether, typically in 15–25% yield. Compound **6** was prepared by iodination of commercially available 4,6-diaminopyrimidine (**4**),[9] while the acetylenes **3** were either obtained from commercial sources or prepared according to various literature procedures.[16]

As was the case with the pteridine series, incorporation of a substituent at the 6-position was found to be important to the potency of the 6,7-disubstituted pyridopyrimidine compounds. Placement of an unsubstituted phenyl group at C-6 yielded an analogue that was about 2-fold more potent than in the unsubstituted case (cf. compounds **16** vs **8**, Table 1). Substituents on the phenyl ring were also key to the potency of these analogues. While not of the same magnitude as in the pteridine series (IC_{50} = 25 nM vs 440 nM),[9] the *p*-dimethylaminophenyl analogue **9** was 4-fold more potent than **8**, and additional increases in potency could be achieved by substituting the phenyl ring with other, particularly more lipophilic, groups. For example, the *p*-bromo analogue **10** had an IC_{50} as low as 20 nM while the *p*-methoxy analogue **12** was 92 nM. Para substitution was preferred over meta substitution as can be seen by comparing the bromo derivatives **10** and **11** and methoxy derivatives **12** and **13**, while 3,4-disubstitution was tolerated as evidenced by analogues **14** and **29**,[17] even when the 3-substituent was methoxy as in analogues **15** and **30**. To further the 6-position SAR, the aromatic ring was extended out from the pyridopyrimidine core by one to three methylene units. It was found that the benzyl analogue (compound **17**, 114 nM) gave similar potencies as the phenyl series while the phenethyl analogue **18** showed only modest potency (433 nM) and the phenylpropyl analogue **19** was greater than 2 μM. Finally, certain alkyl groups could also be placed at the 6-position of the pyridopyrimidine core to give potent AK inhibitors. For example, longer chain groups such as pentyl (compound **20**, 30 nM) were well tolerated while shorter chains and branched alkyl groups such as isobutyl (compound **21**, 200 nM) gave analogues with modest potency.

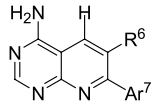


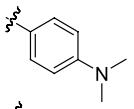
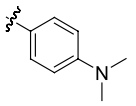
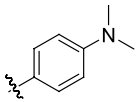
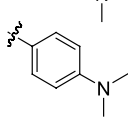
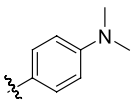
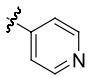
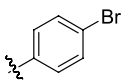
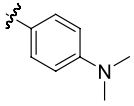
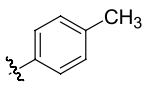
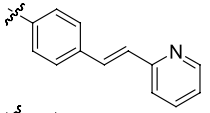
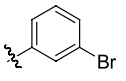
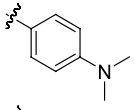
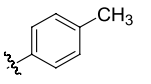
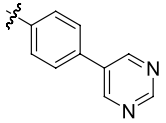
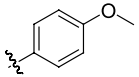
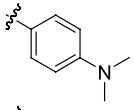
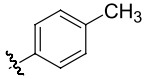
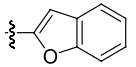
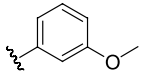
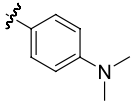
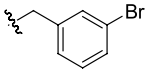
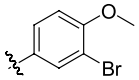
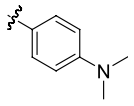
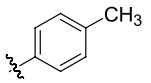
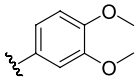
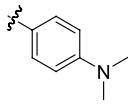
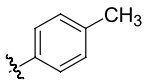
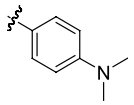
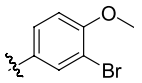
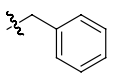
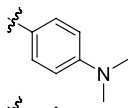
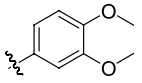
Scheme 1. Reagents and conditions: (a) catecholborane, THF, reflux, 100%; (b) I_2, K_2CO_3, DMF/H_2O, 45 °C, 65%; (c) $(PPh_3)_4Pd$, (aq)Na_2CO_3, THF, reflux, 50–80%; (d) Ar^7CHO, 1,2,4-trichlorobenzene or diphenyl ether, 215–260 °C, air, 15–25%.

A variety of groups were investigated at the 7-position. In addition to the dimethylamino group, a number of other substituted phenyl moieties were tried and, in general, most gave analogues with AK potencies slightly worse to much worse than the dimethylamino group, and usually with much worse solubility. A variety of other aromatic and heteroaromatic groups, some with potential for improving solubility, were also studied. A number of pyridine and pyrimidine analogues such as **22**, **23**, and **24** showed modest potency while the benzofuran analogue **25** and thiazole derivative **26** had potencies near 1 μM. Of all the groups studied at the 7-position, the most striking was the thiophene moiety. While the 3-thiophene group gave an analogue (compound **27**) that was 1.5 times more potent than the corresponding dimethylaminophenyl derivative, it was the 2-thiophene group that consistently yielded compounds with very good AK inhibitory activity, often in the single-digit nanomolar range. For example, thiophene analogues with disubstituted phenyl (**29**), benzyl (**31**), and alkyl (**32**) groups at the 6-position all had very good potencies.

In stark contrast to the other two 4-aminopyridopyrimidine series studied (i.e., 5,7-disubstituted and 5,6,7-trisubstituted), it was found that all of the analogues from the 6,7-disubstituted series of compounds apparently had poor cell membrane permeability as evidenced by the lack of activity in the intact cell assay. In fact, out of more than 110 compounds synthesized the most potent analogue had an IC_{50} of 225 nM (compound **14**). While they share similar calculated physical chemical properties (solubility, clog P, etc), it is this characteristic which makes the 6,7-disubstituted pyridopyrimidine series distinctly different from the 5,7-disubstituted and 5,6,7-trisubstituted pyridopyrimidine series. The latter two series, in particular the 5,7-disubstituted series, were both able to produce analogues with intact cell potencies less than 50 nM. Compounds from these two series, which had single-digit nanomolar potency at the enzyme

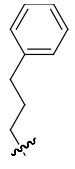
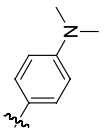
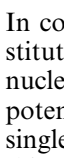
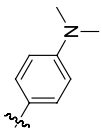
Table 1. In vitro biological activity of 6,7-disubstituted pyridopyrimidines[a]



Compd	R^6	Ar^7	IC_{50} (nM) (enzyme)	IC_{50} (nM) (intact cells)	Compd	R^6	Ar^7	IC_{50} (nM) (enzyme)	IC_{50} (nM) (intact cells)
8	H		773 ± 58	>10,000	**21**			200 ± 50	>1000
9			183 ± 17	367 ± 17	**22**			467 ± 120	4170 ± 930
10			20 ± 3.4	>1000	**23**			115 ± 35	nd
11			70 ± 40	>1000	**24**			180 ± 44	nd
12			92 ± 36	567 ± 233	**25**			>1000	nd
13			>1000	nd	**26**			900 ± 78	nd
14			33 ± 13	225 ± 44	**27**			47 ± 5.8	>1000
15			45 ± 5.0	350 ± 49	**28**			9.0 ± 3.3	800 ± 56
16			350 ± 50	1500 ± 289	**29**			8.0 ± 2.0	375 ± 95
17			114 ± 58	650 ± 50	**30**			38 ± 13	300 ± 100
18			433 ± 120	nd	**31**			3.8 ± 0.71	650 ± 50

(*continued on next page*)

Table 1 (*continued*)

Compd	R^6	Ar^7	IC_{50} (nM) (enzyme)	IC_{50} (nM) (intact cells)	Compd	R^6	Ar^7	IC_{50} (nM) (enzyme)	IC_{50} (nM) (intact cells)
19			2230 ± 958	nd	**32**			11 ± 2.1	767 ± 120
20			30 ± 5.0	700 ± 173					

nd = not determined.
[a] All values are the mean ± SEM of at least three experiments run in triplicate.

that also showed good in vivo efficacy tended to have IC_{50} shifts in the intact cell assay of 5 to 25-fold, while analogues in the 6,7-disubstituted series with single-digit nanomolar IC_{50}'s had shifts of 45 to 170-fold. Selected 6,7-disubstituted compounds were screened in vivo for analgesic activity in the mouse hotplate test.[15] For example, analogues **14**, **15**, **29**, and **30** were dosed at 30 μmol/kg ip and none showed significant effects at both the 30 and 120 min time points. Perhaps this was due to poor solubility or pharmacokinetic properties, or perhaps it was due to the modest intact cell potency (200–400 nM). Therefore, despite very good AK enzyme inhibitory potency, the inability to develop analogues more capable of crossing the cell membrane, as determined by the intact cell AK potency and which presumably would result in in vivo activity, precluded further development of the 6,7-disubstituted 4-aminopyrido[2,3-*d*]pyrimidine series as analgesic agents.

In conclusion, we have developed a series of 6,7-disubstituted 4-aminopyrido[2,3-*d*]pyrimidines as novel non-nucleoside AK inhibitors. These compounds showed potent inhibitory activity at the AK enzyme, some with single-digit nanomolar potency as in the case of the 2-thiophene analogues. A variety of groups at the 6-position such as alkyl, and substituted benzyl and phenyl were evaluated and yielded potent analogues. At the 7-position, a variety of aromatic groups such as substituted phenyl, pyridyl, furyl, and others were studied with the 2-thiophene moiety yielding the most potent analogues, often in the single-digit nanomolar range. In contrast to the 5,7-disubstituted and 5,6,7-trisubstituted pyridopyrimidine series, the 6,7-disubstituted pyridopyrimidine series apparently have poor cell membrane permeability as evidenced by the modest to weak activity in the intact cell assay, and may be the reason analogues from this series were found to be not active in animal pain models such as the mouse hotplate test.

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17. Synthesis of compound **29**: Catecholborane (29.2 mL of 1 M solution in THF) was added to 2-bromo-4-ethynyl-1-methoxybenzene (5.13 g, 24.3 mmol) in THF (100 mL) and the solution was heated to reflux. After 1.5 h an additional 10 mL catecholborane was added and heating was continued for an additional 2 h. The reaction mixture was cooled to ambient temperature, 5-iodo-4,6-diamino-pyrimidine (3.66 g, 15.5 mmol) and $(PPh_3)_4Pd$ (1.5 g, 5.5 mmol) were added, and the mixture was stirred 20 min. 20% aqueous Na_2CO_3 (25 mL) was then added and the reaction mixture was heated to reflux for 15 h. The mixture was then cooled, quenched with water (50 mL), and extracted with EtOAc (3×50 mL). The combined organic extracts were washed with water (2×25 mL), brine (50 mL), and dried over $MgSO_4$. The resulting product was chromatographed (SiO_2, 5% MeOH/CH_2Cl_2 as eluent) to afford 5-[2-(3-bromo-4-methoxyphenyl)-vinyl]-pyrimidine-4,6-diamine (1.09 g; 22% yield). 5-[2-(3-bromo-4-methoxyphenyl)-vinyl]-pyrimidine-4,6-diamine (362 mg, 1.13 mmol), thiophene-2-carboxaldehyde (189 mg, 1.7 mmol), and molecular sieves in diphenylether (10 mL) were heated to reflux for 3 h. The mixture was cooled, diluted with CH_2Cl_2, filtered through Celite and condensed in vacuo. The crude product was purified by flash chromatography (SiO_2, 5% MeOH/CH_2Cl_2 as eluent) followed by trituration with CH_2Cl_2 to afford compound **29** as a pale yellow solid (74 mg; 16% yield). ^1H NMR (DMSO-d_6) δ 8.58 (s, 1H), 8.52 (s, 1H), 8.03 (br s, 2H), 7.69 (m, 2H), 7.42 (dd, 1H, $J_1 = 9$ Hz, $J_2 = 3$ Hz), 7.25 (d, 1H, $J = 9$ Hz), 6.99 (t, 1H, $J = 3$ Hz), 6.73 (d, 1H, $J = 3$ Hz), 3.93 (s, 3H). MS (ESI): m/z 413/415 $(M+H)^+$. Anal. Calcd C, 52.31; H, 3.17; N, 13.56. Found: C, 52.56; H, 3.21; N, 13.69.