Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2071–2074

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Pyridopyrimidine Analogues as Novel Adenosine Kinase Inhibitors

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Received 15 February 2001; accepted 11 May 2001

Abstract—A novel series of pyridopyrimidine analogues **9** was identified as potent adenosine kinase inhibitors based on the SAR and computational studies. Substitution of the C7 position of the pyridopyrimidino core with C2′ substituted pyridino moiety increased the in vivo potency and enhanced oral bioavailability of these adenosine kinase inhibitors.

Adenosine (ADO) is a key homeostatic inhibitory neuromodulator that contributes to endogenous antinociceptive and antiinflammatory responses following tissue injury. Cellular excitability or tissue trauma increases the local tissue levels of extracellular ADO.[1] Extracellular ADO acts on specific cell-surface P1 purinergic receptors (A_1, A_{2A}, A_{2B}, and A_3) proximal to its site of release to exert its homeostatic effects. Due to its extremely short half-life (measured in seconds) in extracellular fluids, the endogenous actions of ADO are localized to the tissue and cellular site where it is released. By blocking the intracellular enzymatic phosphorylation of ADO to adenosine monophosphate by adenosine kinase (AK), selective adenosine kinase inhibitors increase the extracellular concentration of endogenously released adenosine,[2] and therefore enhance its neuroprotective, antinociceptive and antiinflammatory action.[3–5]

Pyridopyrimidine analogues are a novel class of adenosine kinase inhibitors.[6] This class of analogues was originally discovered by HTS (high-throughput screening). Of many potent analogues of this type, compound **9e** (**ABT-702**) was an optimized analogue with good in vitro and in vivo potencies (in vitro: AK (enzyme): $IC_{50} = 1.3$ (± 0.58) nM; AK (intact cell): $IC_{50} = 43.3$ (± 5.7) nM; in vivo: inflammatory hyperalgesia (po): $ED_{50} = 0.7$ μmol/kg).[6] However, its relatively short half-life and moderate oral bioavailability ($t_{1/2} = 0.9$ h; F = 22.6%) needed to be improved. Our goal was to identify analogues with improved half-life and oral bioavailability in our pursuit of a drug development candidate. At the later stages of our lead optimization period, our efforts were mainly focused on several different pyrido-pyrimidine analogues with substituents at C5 and C7 positions. There were several subtypes of C7 substituents.[7] In this letter, we describe our approaches to one of the subtypes, 7-(5′-pyridinyl) analogues, which lead to a potent analogue **9h** (in vitro: AK (enzyme): $IC_{50} = 8.0$ (± 2.0) nM; AK (intact cell): $IC_{50} = 70.9$ (± 19) nM; in vivo: inflammatory hyperalgesia (po): $ED_{50} = 1$ μmol/kg), with better half-life and higher oral bioavailability ($t_{1/2} = 3.6$ h; F = 46.2%) compared to our lead compound **9e**[6] (Fig. 1).

Our earlier observations on this subtype of derivatives indicated that these analogues have very similar adenosine

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0960-894X/01/$ - see front matter
PII: S0960-894X(01)00375-4

kinase inhibitory potency, regardless of the kind of substituents at C2′ of the pyridinyl ring moiety of the C7 pyridopyrimidino core (Fig. 1). The implication of this observation is that the C2′ part of the molecule does not bind to a specific site of the enzyme. In an effort to find out the docking mode of this subtype of analogues within adenosine kinase, we have studied a known crystal structure of adenosine kinase[8] and did molecular modeling with our analogues. These studies suggested that the terminal of the C7 substituent is likely projected outside the binding pocket of the enzyme, which may explain why the terminals of the C7 substituent did not significantly alter AK inhibitory activity of these analogues (Fig. 2). Furthermore, it has been noticed that the majority of the metabolic reactions occur at C2′ substituent. These studies and observations suggest that if we find a suitable group at C2′ to avoid rapid metabolism occurring at that site, we could improve the PK profiles of these analogues without adversely affecting their AKi (adenosine kinase inhibitor) potency. Therefore, a wide range of substituents at the C2′ of the C7 pyridino moiety could be screened to improve half-life and oral bioavailability of analogue **9e** in light our rational (Fig. 2).

After comparing analogues with different **X** linkages at C2′, the nitrogen atom was found to be the best among carbon, oxygen, and sulfur[9] in terms of in vitro and in vivo potencies (Fig. 1). Less metabolic problems were observed when **X** is a nitrogen atom. Analogues with tertiary amines at C2′ demonstrated good correlation between intact cell inhibition values and in vivo efficacy. The synthesis of the nitrogen-linked analogues was simple and straightforward, which allowed access to a variety of analogues in order to compare their physical chemical properties, such as solubility, PK (pharmacokinetic) profiles, as well as in vivo activities.

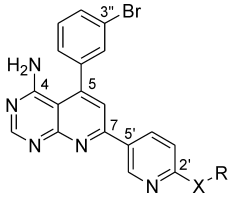


Figure 1.

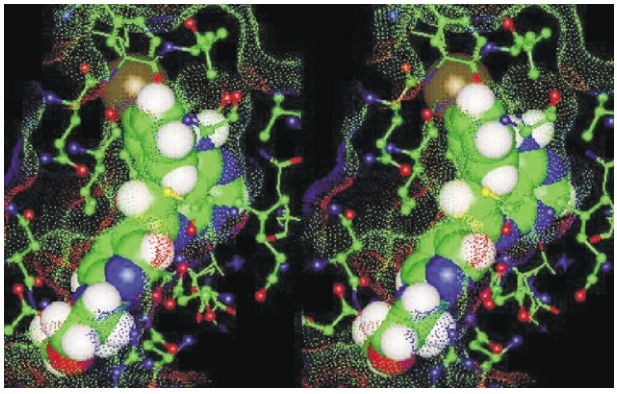
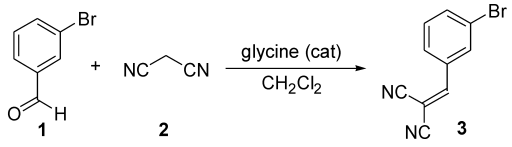


Figure 2. Molecular modeling shown here demonstrates that the oxygen of the morpholino moiety of **9e** (red) is outside the binding pocket of the adenosine kinase.

Table 1 is a summary of typical examples of nitrogen linked tertiary amino analogues. SAR results indicated that analogues with cyclic tertiary amines exhibited better oral activities than those with acyclic tertiary amines. Compound **9a** is a potent AKi in vitro (AK (enzyme): $IC_{50}=4.1$ (±0.020) nM; AK (intact cell): $IC_{50}=42.3$ (±5.6) nM). This compound also potently reduced carrageenan-induced thermal hyperalgesia in the rat ($ED_{50}=1.0$ μmol/kg, ip). Its in vivo potency, however, was diminished when the compound was administrated orally (inflammatory hyperalgesia (po): $ED_{50}>10$ μmol/kg). The lack of oral activity of this analogue (**9a**) was easily explained by its PK profile, which revealed several unidentified metabolites and poor plasma concentration of the parent compound following oral administration in the rat.[10] Cyclic tertiary amine substituents on the C2′ position also yielded potent AK inhibitors. Five-membered ring pyrrolidino analogues **9b** and **9c** had in vivo activity when administered ip. However, these compounds had very low oral bioavailability. Better oral activity and bioavailability were achieved by expanding the five-membered ring to a six-membered ring system at the C2′ position of these analogues (entries 5–8, Table 1). The 4-morpholinyl analogue **9e** (**ABT-702**) was one of the best analogues and showed improved, broad spectrum oral efficacy.[6] Although its plasma half-life still needed to be improved [$t_{1/2}$: 0.9 h (iv)], its oral bioavailability reached a reasonable level (23% in rat and 59% in dog). Moving the oxygen heteroatom of the morpholinyl substituent outside the piperidino ring system gave 4-hydroxy-piperidinyl derivative **9f**, which had improved plasma half-life of 2.1 h, oral bioavailability of 18.6%, and good in vivo activity (inflammatory hyperalgesia in rat (po): $ED_{50}=3$ μmol/kg). Incorporating two oxygen heteroatoms in the spiral ketal derivative **9g** further enhanced the oral bioavailability ($F=44.7\%$), and retained good half-life ($t_{1/2}=2.4$ h) following iv administration. Finally, modification of ketal functionality to a 4-OTHP oxime derivative **9h**, improved plasma half-life to $t_{1/2}=3.6$ h, retained good oral bioavailability ($F=46.2\%$, po) in the rat and improved PK profiles in the dog. Following oral administration, **9h** exhibited an ED_{50} of 1 μmol/kg in reducing carrageenan-induced thermal hyperalgesia in the rat (Table 1).

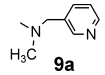
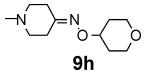
Chemistry

The C2′-derivatives are easily accessible and prepared as shown in Schemes 1, 2, and 3. In the presence of a catalytic amount of glycine, condensation of malono-nitrile with 3-bromobenzaldehyde gave a quantitative yield of 2-(3′-bromophenyl)-1,1-dicyanyl-ethene (**3**) (Scheme 1).

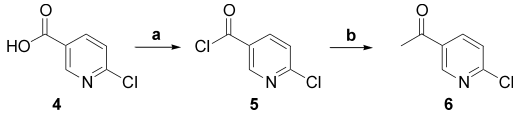


Scheme 1.

Table 1. PK profiles of selected pyridopyrimidine analogues

Entry	C2′-substituent	In vitro (IC_{50}, nM)		In vivo (ED_{50}, μmol/kg)		Pharmacokinetics					
		AK		Inflammatory hyperalgesia		Rat			Dog		
		(enzyme)	(intact cell)	(ip)	(po)	$t_{1/2}$ (iv)	$t_{1/2}$ (po)	F (oral, %) (5.0 μmol/kg)	$t_{1/2}$ (iv)	$t_{1/2}$ (po)	F (oral, %) (5.0 μmol/kg)
1	 **9a**	4.1 (±0.020)	42.3 (±5.6)	1	>10	nf	nf	0.0	nd		nd
2	 **9b**	4.12 (±1.5)	37.7 (±9.6)	6	nd	0.28	nf	0.0	nd		nd
3	 **9c**	3.4 (±2.1)	45.0 (±32)	4	nd	0.41	0.64	4.5	nd		nd
4	 **9d**	1.3 (±1.5)	42.5 (±26)	8	nd	0.25	nf	0.0	nd		nd
5	 **9e**	1.3 (±0.58)	43.3 (±9.6)	0.6	0.7	0.9	nd	22.6	1.2	1.3	61.7
6	 **9f**	1.8 (±0.76)	33.0 (±0.71)	5	3	2.1	nf	18.6	nd		nd
7	 **9g**	1.4 (±1.0)	28.3 (±20)	3	1	2.4	3.6	44.7	4.1	3.4	38.5
8	 **9h**	8.0 (±2.0)	70.9 (±19)	2	1	3.6	4.7	46.2	5.2	3.2	>100

nd, not determined; nf, unable to estimate plasma eliminatiom half-life.

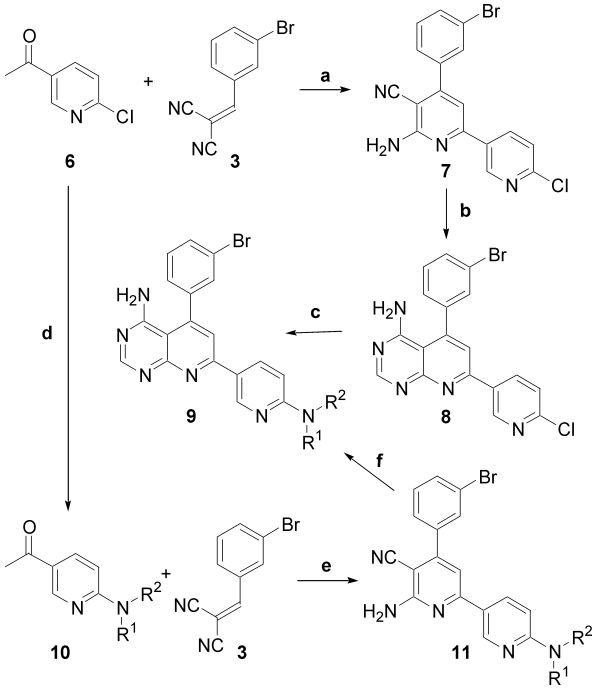


Scheme 2. (a) $(COCl)_2$, DMF (cat), CH_2Cl_2; (b) (1) dimethyl malonate, $MgCl_2$, Et_3N; (2) $DMSO/H_2O$.

3-Acetyl-6-chloropyridine (**6**) was made from the corresponding acid in two steps (Scheme 2).[11]

Condensation of the ketone **6** with 2-(3′-bromophenyl)-1,1-dicyanylethene (**3**) in the presence of ammonium acetate gave the pyridino intermediate **7**.[12] The latter was converted to a key intermediate, **8**, in the presence of trisformamide in formamide. Different primary and secondary amines can be used to generate analogues, which have different amino moieties at the C7′ position. Alternatively, an animo moiety can be put on to the 3-acetyl-6-pyridine (**6**) to give a stable 2-amino-5-acetylpyridine **10**, which is then converted to the final product the same as the previously described routes. The latter approach may increase the chemical yield of the final product, but it is less versatile than the previous approach (Scheme 3).

In conclusion, based on our SAR and computational studies, we have identified several pyridopyrimidinyl



Scheme 3. (a), (e) NH4OAc, dichloroethane; (b), (f) trisformamide, formamide; (c), (d) amine, ethanol.

analogues, which are orally effective in a spectrum of animal pain models with potency and full efficacy comparable to, or superior to morphine.

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7. Research other than that described in this paper will be published in several separate papers.
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9. This portion of the research will be covered by a separate publication.
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