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Synthesis and biological evaluation of pteridine and pyrazolopyrimidine based adenosine kinase inhibitors

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Abstract—Three new approaches have been tested to modify existing pyridopyrimidine and alkynylpyrimidine classes of nonnucleoside adenosine kinase inhibitors **2** and **3**. 4-Amino-substituted pteridines **8a–e** were generally less active than corresponding 5- and 6-substituted pyridopyrimidines **2**. Pyrazolopyrimidine **13c** with $IC_{50} = 7.5$ nM was superior to its open chain alkynylpyrimidine analog **13g** ($IC_{50} = 22$ nM) while pyrrolopyrimidines such as **17a** were inactive.
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Adenosine (ADO) is an extracellular signaling agent within the central and peripheral nervous system.[1] It is released from cells in response to adverse conditions, for example, tissue trauma, pain, seizures, ischemia. Once released, ADO induces protective pharmacological responses upon interaction with specific A_1, A_{2A}, A_{2B}, and A_3 G-protein coupled receptors of the P1 family. However, extremely short half-life of ADO in physiological fluids restricts the protective action of ADO[2] to the tissues and cellular sites where it is released. One of the approaches of increasing the concentration of endogenous ADO and potentiation of its action can be inhibition of ADO metabolizing enzymes. Adenosine kinase (AK) is a key intracellular enzyme regulating intra- and extracellular ADO concentrations.[3] AK inhibitors, therefore, may have therapeutic potential as analgesic and anti-inflammatory agents.

Most of the known early AK inhibitors, for example, 5′-deoxy-5-iodotubercidin[4] **1** (Fig. 1) and several others reported recently[5] have close structural resemblance to the natural ligand ADO. AK inhibitors of this structural class of compounds are commonly referred to as nucleoside-like AK inhibitors. Our group recently reported two classes of nonnucleoside AK inhibitors—pyridopyrimidines **2**[6] and alkynylpyrimidines **3**[7] (Fig. 1). One of the common motifs that both nucleoside and nonnucleoside types of AK inhibitors share is the presence of the lipophilic groups such as I in **1**, aryl and alkyl in **2**, and XAr in **3**. These groups have been shown to be key structural elements responsible for filling a putative hydrophobic pocket of the protein and ensuring effective binding. In this report, we discuss alternative approaches to the design of the AK inhibitors that resulted in structures with different points of attachment of the

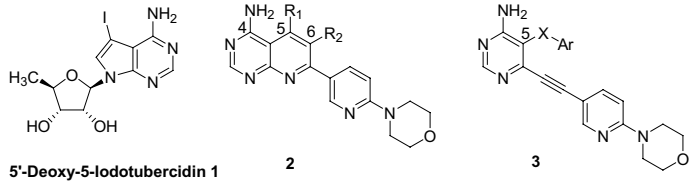

Figure 1. Structures of nucleoside- and nonnucleoside type AK inhibitors.

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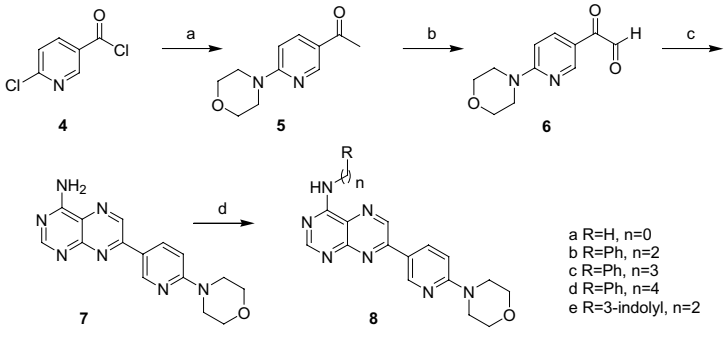
lipophilic group.[8] This in turn led to a more diverse set of AK inhibitors and expanded the scope of SAR in both classes of nonnucleoside series of AK inhibitors.

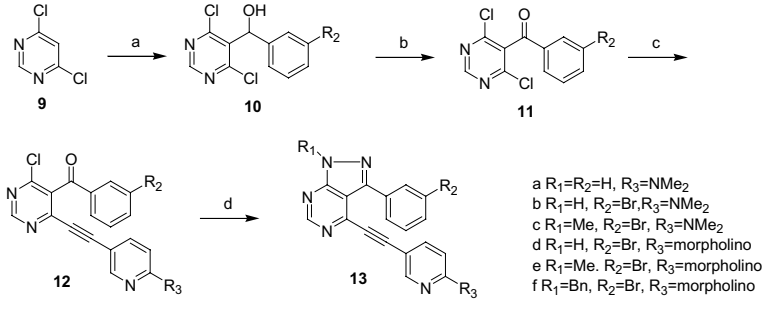
Our objective in the pyridopyrimidine series of AK inhibitors was investigation of SAR at the 4-position. Modifications at two of the other most relevant sites, the 5- and 6-positions of the pyridopyrimidine core, were reported[5] with the 5-position being the optimal point of attachment for the lipophilic fragment. In order to investigate the SAR at the 4-position, we chose to replace the pyridopyrimidine core by its surrogate pteridine core. This strategy was based on the earlier 'SAR by NMR' studies[9] that had shown that the substitution at the 5-position with any moiety, including hydrogen, could potentially cause steric hindrance for any new group introduced in the vicinity of that site, which includes 4-position. A three-step procedure [6a,10] of acylation of dimethyl malonate by commercially available 6-chloro-nicotinoyl chloride **4** (Scheme 1), decarboxylation and replacement of the chloro group by the morpholino group resulted in acetopyridine **5** in 76% overall yield. This compound was oxidized with the selenium dioxide to the ketoaldehyde **6** followed by condensation with 4,5,6-triaminopyrimidine to provide a regiospecifically[11] 7-substituted key precursor **7** with 67% yield for two steps. Target molecules **8a**–**e** with the substitution at the 4-position of the pteridine core were prepared by treatment of **7** with a variety of arylalkylamines in DMSO in the presence of AcOH at 120 °C for 10–12 h. Yields were generally low and variable (7–54%). Longer reaction times resulted in even lower yields.

In the alkynylpyrimidine series of AK inhibitors, the reported structures **3** (Fig. 1) contained lipophilic fragment at the 5-position.[7] To determine the effect of the conformational restriction of that lipophilic group in the known AK inhibitors with an alkynylpyrimidine core, we designed and synthesized pyrazolopyrimidines **13** (Scheme 2). Deprotonation of 4,6-dichloropyrimidine **9** at the 5-position with LDA followed by the reaction with different benzaldehydes provided alcohols **10** (yields 45–65%), which were converted to the ketones **11** by CrO_3 in acetone with generally >90% yield. Sonogashira coupling of **11** with different arylacetylenes in MeCN–Et_3N in the presence of $Pd(PPh_3)_2Cl_2$ and CuI gave monoalkynyl pyrimidines **12** with 36–75% yield. Finally, **12** were reacted with different hydrazines in EtOH or dichloromethane to give the desired pyrazolopyrimidines **13a**–**f**.

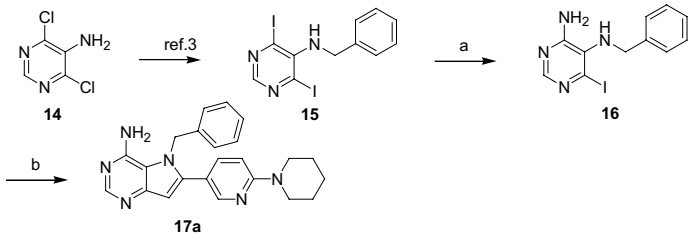
The structure of the pyrrolopyrimidine **17a** (Scheme 3) can be envisaged by closing a ring between the nitrogen of 5-amino group and a carbon on the 6-alkynyl group in the alkynylpyrimidine class of compounds (e.g., **3**, where X = NH, Fig. 1) to form bicyclic core. Iodination and benzylation of 5-amino-4,6-dichloropyrimidine **14** gave **15**[7] in 75% yield. The latter compound was then subjected to ammonolysis in EtOH at 110 °C in a sealed tube for 24 h to obtain **16** in 72% yield. Reaction of **16** with 4-(5-ethynyl-pyridin-2-yl)-morpholine[6] under Sonogashira conditions provided the pyrrolopyrimidine **17a** in 67% yield in a two-step in situ process of the cross-coupling followed by the intramolecular hydroamination. This sequential reaction represents the



Scheme 1. Reagents and conditions: (a) (1) dimethyl malonate, $MgCl_2$, Et_3N; (2) DMSO–H_2O, 150 °C, 80% for two steps; (3) morpholine, EtOH, 95%; (b) (1) SeO_2, 1,4-dioxane–H_2O; (c) 4,5,6-triaminopyrimidine, $BaCl_2$, 67% for two steps; (d) arylalkyl amine, DMSO, AcOH, 120 °C.



Scheme 2. Reagents and conditions: (a) LDA, −78 °C, THF, then benzaldehyde; (b) CrO_3, acetone, 2 h, rt; (c) 4-(5-ethynyl-pyridin-2-yl)-morpholine, $Pd(PPh_3)_2Cl_2$, CuI, MeCN–Et_3N (1:1), 60 °C, 6 h; (d) $RNHNH_2$, CH_2Cl_2 (when R = Me), EtOH (when R = H, Bn).



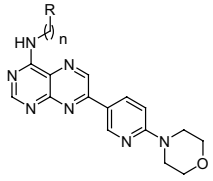
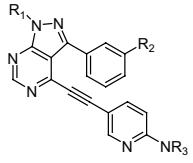
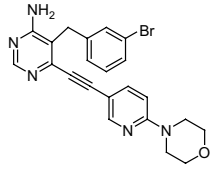
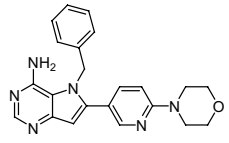
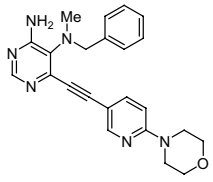
Scheme 3. Reagents and conditions: (a) NH_3, EtOH, 24 h, 110 °C, sealed tube, 72%; (b) 4-(5-ethynyl-pyridin-2-yl)-morpholine, $Pd(PPh_3)_2Cl_2$, CuI, MeCN–Et_3N (1:1), rt, 1.5 h, 67%.

application of the Larock's indole synthesis procedure[12] to the heteroaromatic systems such as the pyrimidine **16**.

The results of in vitro inhibition of the cytosolic AK[13] by these novel compounds are summarized in Table 1. Substitution at the 4-position in the pteridine **8a** resulted in compounds **8b–e** that exhibited 1.5–6.5-fold increase in AK inhibitory potency, in comparison to the unsubstituted compound **8a**. The length of the alkyl group linking the lipophilic aryl group with the 4-amino group of the pteridine core does not seem to significantly alter the AK inhibitory activity as the largest difference in a series of phenethyl-, phenylpropyl-, and phenylbutyl-substituted compounds **8b–d** is only about 1.5-fold (120 nM for **8b** vs 72 nM for **8c**). Although the in vitro potency of the 4-substituted pteridines **8b–e** is lower than some of the best compounds in the 5-substituted pyridopyrimidines of general structure **2**,[6] the present data suggested the existence of acceptable tolerance for the 4-substitution and offered the possibility of approaching the putative hydrophobic pocket from the 4-position of the pyrimidine ring.

Conformationally rigid analogs of the alkynylpyrimidine AK inhibitors of the general structure **3** were made by cyclization of the benzylic carbon with 4-amino nitrogen of the pyrimidine core. Inspection of the biological data on the resulting pyrazolopyrimidines **13a–f** revealed that the structural constraints were not detrimental and may be beneficial for AK inhibition. Thus, compounds **13c** and **13d** have IC_{50} values of 7.5 and 22 nM that are better or equal to the corresponding inhibition by open chain analog **13g** ($IC_{50} = 22$ nM).[7] The benzyl group in the pyrazole fragment of **13f** greatly reduced the AK inhibitory potency (IC_{50} >1000 nM) in comparison with the unsubstituted analog **13d** ($IC_{50} = 22$ nM). This could be due to the competition between the benzyl group and the 3-bromophenyl group to occupy the hydrophobic binding site. It is more difficult to explain the trends in the pairs **13b** and **13c** versus **13d** and **13e**. The structural difference between these pairs is dimethylamino group versus morpholino group as a part of 7-substituent. These amino groups have been shown earlier to be similar in their effects on AK inhibition.[6a] However, in the first pair, methylation of the pyrazole ring increases the AK inhibition (7.5 nM for **13c** vs 75 nM for **13b**), while in the second pair, the same exercise decreases the inhibition (82 nM for **13e** vs 22 nM for **13d**).[14]

Table 1. In vitro activity of AK inhibitors in cytosolic assay

Compounds		AK inhibition IC_{50} (nM)[a]
	**8a** R = H, $n = 0$	460 ± 45
	8b R = Ph, $n = 2$	120 ± 33
	8c R = Ph, $n = 3$	72 ± 23
	8d R = Ph, $n = 4$	82 ± 17
	8e R = 3-indolyl, $n = 2$	300 ± 65
	**13a** $R_1 = R_2 = H$, $R_3 = NMe_2$	100 ± 34
	13b $R_1 = H$, $R_2 = Br$, $R_3 = NMe_2$	75 ± 15
	13c $R_1 = Me$, $R_2 = Br$, $R_3 = NMe_2$	7.5 ± 1.5
	13d $R_1 = H$, $R_2 = Br$, $R_3 =$ morpholino	22 ± 15
	13e $R_1 = Me$, $R_2 = Br$, $R_3 =$ morpholino	82 ± 13
	13f $R_1 =$ Bn, $R_2 = Br$, $R_3 =$ morpholino	>1000
	**13g**	22 ± 8
	**17a**	>1000
	**17b**	30 ± 4

[a] The data represent mean ± SEM from at least three determinations.

The closest ring-opened analog for the pyrrolopyrimidine **17a** would be 6-alkynylpyrimidine **17b**[7] with IC_{50}

value of 30 nM. *N*-Demethylation of this compound with subsequent ring closing by intramolecular hydroamination of the triple bond yields the compound **17a** that is virtually inactive (IC_{50} >1000 nM). This reinforces the importance of a rigid linear trajectory of the alkynyl substituent in the 6-position of pyrimidines like **3** for the optimum inhibition of the enzyme.[15]

In summary, we have investigated three new approaches of modifying existing pyridopyrimidine and alkynylpyrimidine AK inhibitors. It was shown that substitution at the 4-position of the pteridine core, as a surrogate for the pyridopyrimidine, resulted in compounds **8a**–**e** which, while potent, were generally inferior to the previously reported best examples of the pyridopyrimidine class of compounds having substitutions at 5- and 6-positions. In the case of the alkynylpyrimidine AK inhibitors, restriction of the flexibility of the lipophilic group at the 5-position by forming fused pyrazole ring between the nitrogen of the 4-amino group and the benzylic carbon of 5-benzyl group can be beneficial as compounds **13c** ($IC_{50} = 7.5$ nM) and **13d** ($IC_{50} = 22$ nM) displayed increased or equal AK inhibition in comparison with ring-opened analog **13g** ($IC_{50} = 22$ nM). In contrast, the pyrrole ring formation between the nitrogen of the 5-substituent and the alkynyl carbon at the 6-position yielded compound **17a** that was significantly less active (IC_{50} >1000 nM) than corresponding ring-opened molecule **17b** ($IC_{50} = 30$ nM). This can potentially be explained by the loss of specific binding of the morpholinopyridyl substituent due to an incorrect steric direction.

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