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Discovery and optimization of 2-aryl oxazolo-pyrimidines as adenosine kinase inhibitors using liquid phase parallel synthesis

M. Bauser,[a,*] G. Delapierre,[a,†] M. Hauswald,[a] T. Flessner,[a] D. D'Urso,[b] A. Hermann,[b] B. Beyreuther,[b] J. De Vry,[b] P. Spreyer,[b] E. Reissmüller[b] and H. Meier[a]

[a]*BAYER AG, Bayer Health Care, Division Pharma, Medicinal Chemistry, Germany*
[b]*BAYER AG, Bayer Health Care, Division Pharma, CNS Research, Germany*

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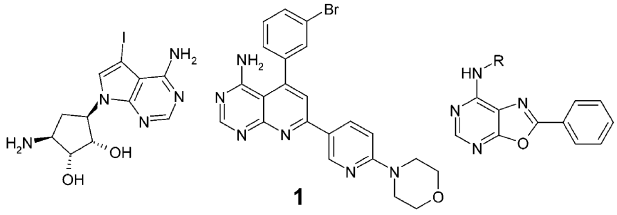
Abstract—Adenosine kinase inhibition is an attractive therapeutic approach for several conditions for example, neurodegeneration, seizures, ischemia, inflammation and pain. Several nucleosidic and non-nucleosidic inhibitors are available. Using a virtual screening approach, we have discovered that 2-aryl oxazolo-pyrimidines are adenosine kinase inhibitors. Subsequent high throughput derivatization enabled the optimization of this new inhibitor chemotype resulting in highly potent derivatives. A variety of analogues were produced by applying liquid phase parallel synthesis to vary the 7-amino residues as well as the 2-aryl moiety.

Adenosine acts as an extracellular signaling molecule, modulating cellular activity and local nutrition supply. It binds to specific purinergic receptors,[1] several subtypes of which are described in the literature (A_1, A_{2a}, A_{2b} and A_3).[2] Adenosine levels are increased under adverse conditions due to the intra- and extracellular production of adenosine from adenosine phosphates. Strong evidence suggests that this protective pathway is involved in pathological processes including neurodegeneration, seizures, ischemia, inflammation and pain.[3] Adenosine produces analgesic effects if applied intrathecally[4] and mice lacking the A_1 receptor exhibit hyperalgesia.[5] However, the short plasma half-life of adenosine and possible side effects of systemic treatment with A_1 agonists limits their therapeutic use. Alternatively, analgesic effects can be achieved by increasing local extracellular adenosine levels by inhibiting the intracellular metabolizing enzyme adenosine kinase, which is responsible for phosphorylating adenosine to AMP, inside the cell. Since adenosine uptake is driven by its concentration gradient, intracellular inhibition of adenosine kinase decreases cellular re-uptake of adenosine, leading to increased extracellular adenosine levels. This approach, which acts selectively in tissues with increased adenosine concentrations, offers some benefits over the use of purinergic receptor agonists, because purinergic receptors are widespread and undesirable side effects could result from applying systemic treatments. Consequently, adenosine kinase and its inhibitors continue to be studied quite intensively.

A high-resolution crystal structure of the human enzyme[6] has been published and several inhibitors, nucleosidic[7] and non-nucleosidic,[8] are known (Scheme 1). However, none of the currently available inhibitors have proceeded to an advanced development stage so far.

Therefore we were interested in finding new non-nucleosidic chemotypes of adenosine kinase inhibitors. Based on the available inhibitors (Scheme 1), the



Scheme 1. Structures of known nucleosidic and non-nucleosidic adenosine kinase inhibitors and the proposed general structure of the oxazolopyrimidine structure.

* Corresponding author. Tel.: +49-(0)202-364981; e-mail: marcus.bauser.mb@bayer-ag.de
† CEA/LETI, Biology and Health Care Systems Department, Grenoble, France.

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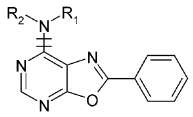
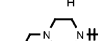
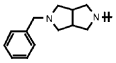
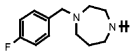
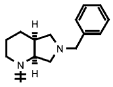
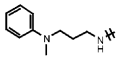
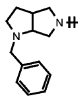
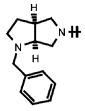
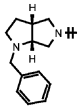
natural substrate, and the enzyme X-ray structure, a virtual screening approach led to the proposal of 2-aryl 7-amino-oxazolo[5,4-*d*]pyrimidines as possible framework for new lead structures.

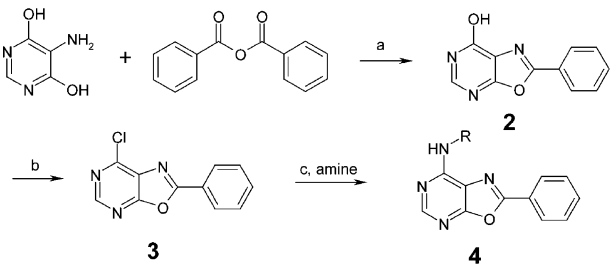
The target oxazolo-pyrimidines can be synthesized via two major routes, either starting from 5-amino 4-cyano oxazoles,[9] or in three steps from commercially available 5-amino-4,6-dihydroxy-pyrimidine[10] (Scheme 2). We found the second route to be well suited for solution phase parallel synthesis. 5-Amino-4,6-dihydroxy-pyrimidine was condensed with acid anhydrides, for example, benzoic anhydride, at elevated temperature to form 2-phenyloxazolo[5,4-*d*]pyrimidin-7-ones **2** in high yield and purity. These intermediates were then transformed quantitatively into the corresponding chloro derivatives **3** on treatment with phosphorous oxychloride at reflux temperature. Displacement of the chloro substituent was easily accomplished by heating an amine and **3** in the presence of a base in DMF. The carboxylic acids and amines required to probe the effect of the substituents at the 2 and 7 position of the oxazolo-pyrimidine scaffold are readily available in large numbers and high diversity. The chloro derivatives of type **3** were synthesized on a multi-gram scale. Derivatives **4** were produced in a library format by exchanging the chlorine substituent with amines. We were able to introduce primary and secondary amines including sterically hindered amines, for example, *tert*-butyl amine, and also anilines in yields varying from 20 to 90%. Library synthesis was performed in special glass micro titer plates, enabling efficient mixing and heating. Workup was performed by parallel filtration followed by preparative HPLC-MS.[11] In this manner, more than 800 compounds were made by varying the amine in position 7 while keeping position 2 constant as phenyl. These compounds were tested for adenosine kinase inhibition in an assay similar to previously described methods.[12] The activity found for some derivatives (Table 1) provided support for our pharmacophore model. Specifically, the screening results indicate that an additional aryl moiety in the amine residue at position 7 is necessary; simple alkyl derivatives **4a**,**b** were inactive. Moreover, in active compounds the aryl moiety must be connected to the scaffold by a spacer with a well-defined length and geometry.

The benzyl piperazine **4c**, the bis-pyrrolidine **4d** were as well as 4-fluoro benzyl homopiperazine **4e** were inactive. Benzyl pyrrolo-piperidine **4f** was slightly active. The most potent compounds in this series were the simple open chain derivatives **4g** and **h** and the racemic benzyl bis-pyrrolidine **4k**.[13] Preparation of the enantiomeres of **4k**, using both enantiomeres[14] of the benzyl bis-pyrrolidine resulted in derivatives **4l** and **m**, which exhibited a difference in activity of one order of magnitude, emphasizing the importance of the proper geometry for activity. Subsequently, variation of the spacer length of the more simple open chain derivatives, showed that activity was lost with shorter chains between scaffold and aryl moiety **4i**,**j**. These results provide an illustration of the principle that synthesis of larger compound libraries can be useful for generating lead structures in early project stages using diverse starting materials. To further optimize this compound class, the racemic bis-pyrrolidine residue was kept constant as shown in **4k** and the phenyl moiety in position 2 was varied. We added amino and bromo functionalities at the aryl residue, thereby enabling further parallel derivatization using amide bond forming and Suzuki coupling reactions (Scheme 3).

Table 1. AKI inhibition by derivatives of **3**



	Amine	AKI IC_{50} [nM]		Amine	AKI IC_{50} [nM]
1	ABT-702	10 ± 3	**4a**		> 10.000#
4b		> 10.000#	**4c**		> 10.000#
4d		> 10.000#	**4e**		> 1.000#
4f		1.000#	**4g**		270 ± 80
4h		230 ± 60	**4i**		> 10.000#
4j		> 10.000#	**4k**		200 ± 18
4l		1.500 ± 120	**4m**		120 ± 6

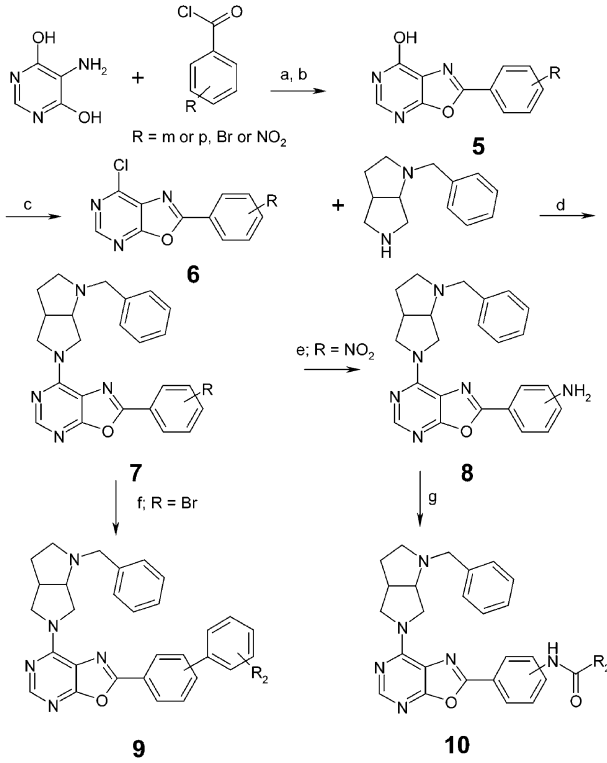


Scheme 2. Reagents and conditions: (a) neat, 140 °C, 5 h; (b) $POCl_3$, reflux, 6 h; (c) (d) amine, DMF, Et_3N, 110 °C, 16 h.

The derivatives of type **5** were prepared by using the corresponding benzoic acid halides. The first step, acylation of the amino group in 5-amino 4,6-dihydroxy pyrimidine, is followed by cyclization in polyphosphoric acid at elevated temperature. The resulting 7-hydroxy oxazolo-pyrimidines are transformed via phosphorous oxychloride treatment to the corresponding chloro derivatives **6**. The benzyl bis-pyrrolidine residue is introduced upon treating the chloro derivatives **6** with the corresponding amine in DMF in the presence of base. The nitro derivatives of type **7** are reduced using catalytic hydrogenation. Parallel substitution of bro-

mine using Suzuki coupling reactions was performed in dioxane/water using $Pd(PPh_3)_4$ as the palladium source. Amide bond formation was accomplished using carboxylic acids and standard coupling reagents or acid halides in the presence of base. Over 100 amides and more than 60 biaryl compounds were prepared in a library and tested for inhibition of adenosine kinase activity and results can be summarized as follows. Substituting the aryl ring in *para* position led to compounds with slightly better activity than the corresponding *meta* series. Substituting at the *para* or *meta* position with amides gave compounds of higher potency than did substitution at this position with aromatic rings. In the series produced by adding aryl rings through the Suzuki reaction, only heteroaryl substitution in the *para* position, for example, **9a**, resulted in derivatives with notably higher activity compared to the precursors **7a**,**b** and the unsubstituted phenyl derivative **4k** (Table 2).

Substitution with a substituted phenyl led to inactive compounds, for example, **9b**. Adding benzoic acids on the amine **8** resulted in poor activity, only smaller substituents like fluorine being tolerated on the aromatic ring as in **10c**,**d**. The benzamide itself was the most potent example amoung the benzoyl series. However phenylacetic acid derivatives are almost as potent as phenyl derivatives **10e** and **f**. Derivatives **10i**–**l** were twice as potent than the corresponding precursor **8** and five times more potent than the unsubstituted 2-phenyl oxazolo-pyrimidine **4k**. Building on the activity of aniline **8a**, the 5-aryl moiety was converted to pyridines **11a**–**c** in order to present a more basic functionality.

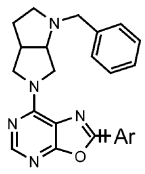


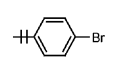
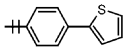
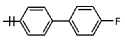
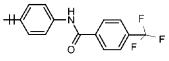
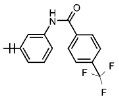
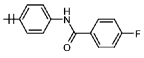
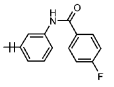
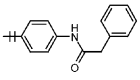
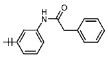
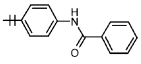
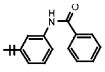
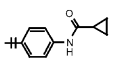
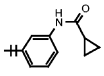
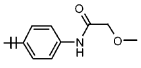
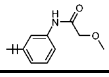
Scheme 3. Reagents and conditions: (a) Pyridine, 50 °C, 16 h; (b) PPA, 100 °C, 6 h; (c) $POCl_3$, reflux, 5 h; (d) DMF, Et_3N, 110 °C, 16 h; (e) Pd/C H_2, MeOH, 16 h; (f) boronic acid, $Pd(PPh_3)_4$, K_2CO_3, dioxane/water, 80 °C, 16 h; (g) carboxylic acid, TBTU, DMF, Et_3N, 16 h.

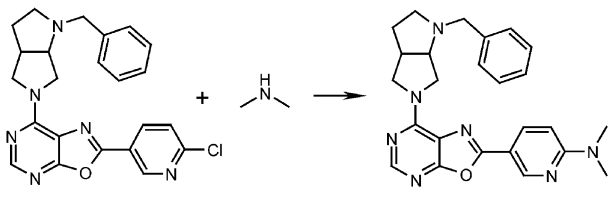
Those were prepared using the procedure depicted in Scheme 3 using pyridyl-carboxylic chlorides. Additionally, in this series we investigated amino-pyridines easily prepared via the chloro intermediate **11** (Scheme 4).

While the 4-pyridyl derivative **11a**, disappointingly showed no activity, results from the 3-pyridyl derivatives **11b**/**11c** indicated room for optimization in this sub-series. This was nicely confirmed by the *N*,*N*-dimethyl derivative **12** leading to the first derivative in our program with a ≤ 10 nanomolar IC_{50} value for adenosine

Table 2. AK Inhibition of 2-aryl substituted 7-benzyl bispyrrolidino oxazolo-pyrimidines

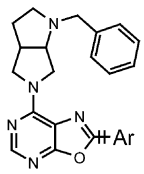


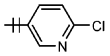
	Aryl residue	AKI IC_{50} [nM]		Aryl residue	AKI IC_{50} [nM]
1	ABT 702	10 ± 3			
7a		240/140	**7b**		260 / 60
9a		90 ± 40	**9b**		> 10.000#
8a		50 ± 6	**8b**		n.d.
10a		> 10.000#	**10b**		> 10.000#
10c		100/130	**10d**		190/200
10e		70/50	**10f**		150/210
10g		70/50	**10h**		50/90
10i		30/30	**10j**		60/50
10k		40/40	**10l**		40/30



Scheme 4. Reagents and conditions: Excess dimethylamine, 2M in THF, rt, 40 h.

Table 3. AK Inhibition of 2-pyridyl substituted 7-benzyl bispyrrolidino oxazolo-pyrimidines



	Aryl residue	AKI IC_{50} [nM]		Aryl residue	AKI IC_{50} [nM]
1	ABT 702	10 ± 3			
11a		$>1.000^{\#}$	**11b**		90/130
11c		$70^{\#}$	**12**		10/6

kinase inhibiton, comparing favorably with the reference ABT 702 **1** in our assay (Table 3).

In summary, we have discovered that oxazolo pyrimidines are a novel class of structurally novel adenosine kinase inhibitors. High throughput derivatization of the oxazolo-pyrimidine scaffold was performed using liquid phase parallel synthesis techniques. This led to the discovery of **12**, an adenosine kinase inhibitor with a ≤ 10 nanomolar IC_{50} value.

Acknowledgements

We thank Dr. R. Grosser for separating **4k** into the enantiomeres, A. Gräwinger, L. Jansen, R. Müller, C. Oschwald, T.-O. Repp, D. Schneider, C. Wilkop, D. Zehner, I. Pluschkell, J. Micksch, D. Hüttebräuker for preparing and purifying the compound libraries and I. Flocke for performing the AK assay.

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