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Design and preparation of 2-benzamido-pyrimidines as inhibitors of IKK

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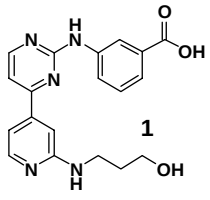
Abstract—The design, synthesis, and the biological evaluation of 2-benzamido-pyrimidines as novel IKK inhibitors are described. By optimization of the lead compound **3**, compounds **16** and **24** are identified as good inhibitors of IKK2 with IC_{50} values of 40 and 25 nM, respectively. Compound **16** also demonstrated significant in vivo activity in an acute model of cytokine release.

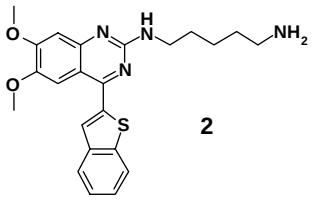
Over the past few years, IκB kinase (IKK) emerged as a prime target for the development of novel anti-rheumatic and anti-inflammatory drugs.[1] IKK is part of a high molecular weight protein complex which consists of three subunits, the structurally related kinases IKK1 and IKK2, and the regulatory subunit NEMO (IKKγ). However, in studies using genetic mutants it was shown that IKK2 is the essential kinase for the production of pro-inflammatory cytokines such as IL-1β and TNFα.[2] Recently, several classes of low-molecular weight inhibitors of IKK2 were disclosed, some of which demonstrate impressive activity in animal models.[3] These findings further confirm the importance of IKK2 as a key player in the transduction of pro-inflammatory signals.

In this paper, we present our own efforts in the discovery of novel and selective inhibitors of IKK, particularly of IKK2. By screening the Novartis compound archive, several hits with typical kinase inhibitor motifs (2-anilino-pyrimidines and 2,4-disubstituted quinazolines) were found. Compounds **1** and **2** (depicted below) are typical prototypes and represent two of the most interesting hits. Both of them are active in the micromolar range.



1

IC_{50} IKK2: 15 μM



2

IC_{50} IKK2: 30 μM

During our first attempts to develop a structure–activity relationship (SAR) and to identify the key features that would drive the inhibition, we quickly realized that hybrid molecules between **1** and **2**, combining structural features of the two parent hits, led to a dramatic improvement in potency (Table 1).

The *para*-carboxyl-substituted anilino-pyrimidine **3** was the most potent representative of this small series. It was slightly more active than the *meta*- but clearly favored over the *ortho*-derivative (compounds **4** and **5**). Extension by one carbon to the *para*-acetic acid **6** did not improve the potency against IKK2.

Interestingly, compound **3** was only weakly active on the complete IKK complex isolated from HeLa cells,[4]

Keywords: 2-Benzamido-pyrimidines; IKK inhibitors; IKK2; In vivo activity.
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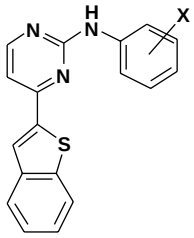
Table 1. Structure–activity relationship of carboxyl-substituted 2-anilino-pyrimidines



Compound	X	IKK2 IC_{50} (μM)	IKK1 IC_{50} (μM)	IKK complex IC_{50} (μM)
3	*p*-CO_2H	0.6	5	18
4	*m*-CO_2H	1	n.d.	n.d.
5	*o*-CO_2H	>100	>100	>100
6	*p*-CH_2CO_2H	2.2	n.d.	n.d.

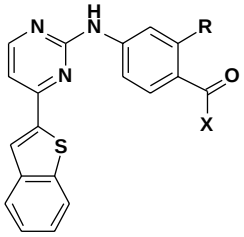
despite the fact that it inhibited both isoforms of the IKK enzymes.

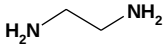
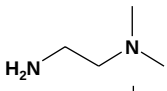
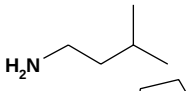
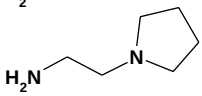
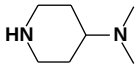
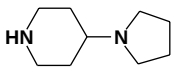
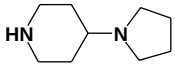
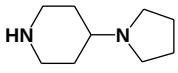
When we replaced the carboxyl group in **3** by an amide functionality (Table 2), it became apparent that a negatively charged residue was not mandatory since the amide **10** completely retained activity against IKK2. Based on this initial result we decided to prepare a small library of 2-anilino-pyrimidines with *para*-substituted amino-benzamides. The synthetic strategy is outlined in Scheme 1.

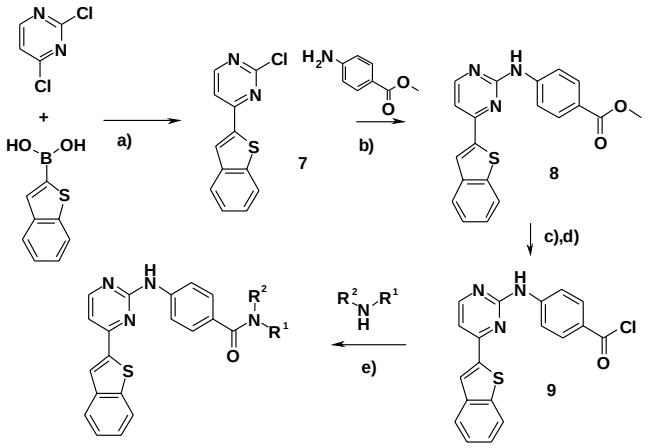
Suzuki coupling[5] of 2,4-dichloropyrimidine and 2-benzothiophene boronic acid resulted in intermediate **7**, which was treated with methyl 4-amino-benzoate at high temperature. The resulting ester **8** was saponified with sodium hydroxide and then treated with thionyl chloride to give key intermediate **9**. Finally, reaction of compound **9** with a set of aliphatic amines in the presence of triethylamine produced the desired benzamides. Data for selected compounds are summarized in Table 2.

Generally, compounds with an additional basic amino group in the amide portion were more active than the neutral inhibitors **10** and **13**. Tertiary (and more basic) amines were better than primary ones (cf. **12** and **14** vs **11**). Finally, the replacement of the flexible ethyl chain in **12** or **14** by the conformationally restricted piperidine ring (cf. **15** and **16**) resulted in a 10-fold increase in potency. The tertiary amines, such as **15** and **16**, were

Table 2. Structure–activity relationship of *para*-amino benzamides



Compound	X	R	IKK2 IC_{50} (μM)	IKK1 IC_{50} (μM)	IKK complex IC_{50} (μM)
10		H	0.9	3.8	8.5
11		H	0.6	3.7	4.4
12		H	0.2	1.2	0.3
13		H	>100	n.d.	n.d.
14		H	0.4	1.2	0.5
15		H	0.04	0.15	0.08
16		H	0.04	0.2	0.07
17		Cl	0.09	0.4	0.25
18		OMe	0.05	0.1	0.15



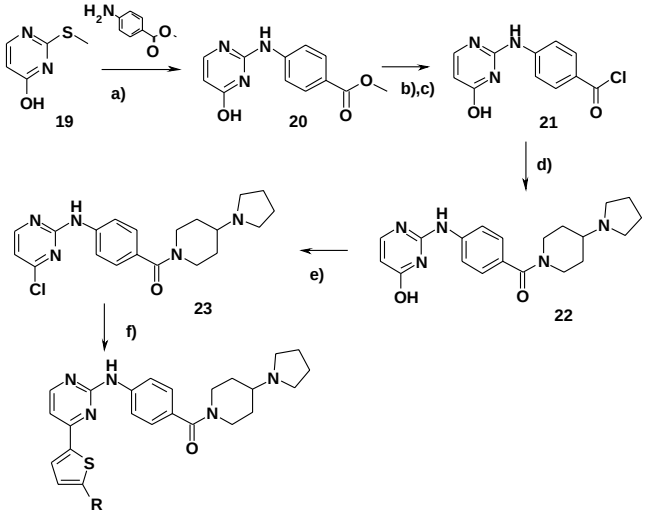
Scheme 1. Synthesis of 2-benzamido-4-benzothiophenyl-pyrimidines. Reagents and conditions: (a) $Pd[P(Ph)_3]_4$; DME, 1 N $NaHCO_3$; (b) neat, 200 °C; (c) 1 N NaOH; (d) $SOCl_2$; (e) 'amine', THF, NEt_3.

active in the low-nanomolar range and were the most potent compounds of this series. Further substitution of the phenyl ring of the benzamide moiety did not improve activity (compounds **17** and **18**).

Most of the benzamides were moderately selective for IKK2 (over IKK1) but, in contrast to the carboxylic acid **3**, showed comparable activity against the complete IKK complex.

To further extend the SAR of the benzamide series, we next replaced the benzothiophene group by a variety of structurally close, but more polar, thiophenes. The synthesis is outlined in Scheme 2.

Treatment of 2-methylsulfanyl-pyrimidin-4-ol, **19**, with methyl *para*-amino-benzoate in 1,3-dimethyl-imidazolidin-2-one (DMEU) at 170 °C gave anilino-pyrimidine **20**. Ester hydrolysis of **20** followed by treatment with



Scheme 2. Synthesis of 2-anilino-4-thiophenyl-pyrimidines. Reagents and conditions: (a) DMEU, 170 °C; (b) 1 N NaOH; (c) $SOCl_2$; (d) 4-(1-pyrrolidinyl)-piperidine, NEt_3; (e) $POCl_3$, 4 N HCl in dioxane; (f) $Pd[P(Ph)_3]_4$, '2-thiophenyl-boronic acid'.

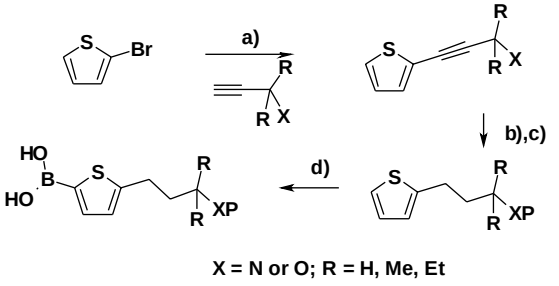
thionyl chloride produced the corresponding acid chloride **21**, which was easily converted into amide **22**. Subsequent treatment with phosphoroxy chloride resulted in chloro-pyrimidine **23**. The desired new pyrimidines were finally obtained by a Suzuki coupling between **23** and a variety of substituted 2-thiophenyl boronic acids. These novel boronic acids were synthesized according to the general strategy outlined in Scheme 3.

The SAR for this series of compounds is summarized in Table 3. In general, replacement of the benzothiophene group in **16** by 5-substituted thiophenes was well tolerated without loss of activity. Saturated side chains appeared to be slightly favored over the more rigid propargyl chain (cf. **28** vs **27**). Hydrogen-bond donors like hydroxyl (**24**) or amino groups (**28**) had a beneficial effect on the potency. Both, methylation, as in **25**, or acetylation, as in **29**, yielded less potent compounds. Remarkably, the loss of activity of compound **29** was much more dramatic for IKK1 than for IKK2, an observation that we could not explain and we have not followed up so far. Introduction of steric bulk in the form of geminal dialkyl groups (cf. **26**, **28** or **30**) was well tolerated, indicating that the binding pocket provides enough space to accommodate bulky substituents.[7]

In order to determine whether blockade of IKK2 resulted in inhibition of relevant downstream events, selected compounds were tested in a cellular assay assessing the functional consequence of IKK inhibition as detected by the blockade of IκBα degradation.[8] In this cellular assay, the potency of the compounds was about 10- to 40-fold lower than in the cell-free kinase assays (see Table 4), presumably reflecting incomplete penetration of the compounds into the cells or different ATP concentrations in the cells compared to the cell-free assay system.

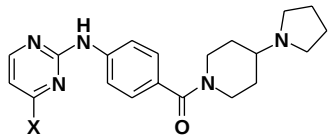
Generally, the SAR in this cellular assay correlated nicely with the SAR in the IKK2 assay.

To determine the selectivity of our compounds on a cellular level, we tested the same set of compounds (Table 4) for their ability to interfere with TNFα-stimulated expression of the adhesion molecules E-selectin,



Scheme 3. Synthesis of substituted thiophene boronic acids. Reagents and conditions: (a) 'Sonogashira[6]' coupling, $PdCl_2[P(Ph)_3]_2$, Cu(I)I, NEt_3, DMF; (b) H_2/Pd/C; (c) introduction of protecting group 'P'; (d) LDA, $B(OEt)_3$.

Table 3. Structure–activity relationship of benzthiophene replacements



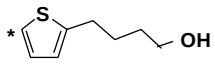
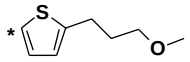
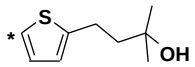
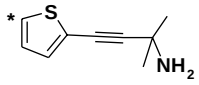
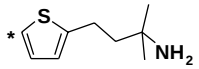
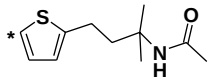
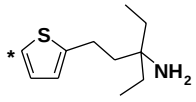
Compound	X	IKK2 IC_{50} (μM)	IKK1 IC_{50} (μM)	IKK complex IC_{50} (μM)
24		0.025	0.5	0.04
25		0.1	1.0	0.1
26		0.05	0.7	0.05
27		0.15	0.5	0.2
28		0.04	0.03	0.04
29		0.15	30.0	0.8
30		0.06	0.03	0.03

Table 4. Cellular profile of selected compounds

Compound	IκB degradation (μM)	E-Sel (μM)	ICAM (μM)	VCAM (μM)	β2M (μM)	HLA-DR (μM)
3	100	50	50	50	100	100
10	20	2	2	2	10	0.25
12	5	0.5	0.5	0.5	2	0.5
15	2	0.3	0.4	0.4	2	2
16	1	0.5	0.3	0.3	2	2
24	2	0.2	0.2	0.2	5	2.5
25	1	0.4	0.6	0.5	5	2
26	2	0.45	0.5	0.6	5	3

ICAM-1, and VCAM-1 in HUVEC cells.[9] In addition, the compounds were also tested for their effects on IFNγ-stimulated expression of the MHC molecules β2 microglobulin and HLA-DR, both events which are not dependent on IKK activity.[10,11] As illustrated in Table 4, our compounds inhibited TNFα-induced adhesion molecule expression in a potency range similar to the IκBα degradation. Although compounds generally showed activity in the IFNγ-induced expression of β2 microglobulin or HLA-DR, their potency in these assays was 4- to 10-fold (example **16**, **25**, and **26**) or even 10- to 15-fold (example **24**) weaker. These data demonstrate that our IKK2 inhibitors have an effect on downstream gene expression, however, on the cellular level the selectivity was modest.

Compound **16** was also tested in two animal models. First, its efficacy to inhibit TNFα release into plasma upon LPS-challenge in the rat was determined. The compound was dosed sc (30 mg/kg) or orally (30 mg/kg) 1 h prior to the LPS-challenge. Four hours after the challenge, plasma was collected and the systemic TNFα levels were analyzed using a commercially available ELISA kit. Both routes of administration of inhibitor **16** at the indicated dose resulted in a significant inhibition of 86% (sc) and 75% (p.o.).[12] In a second experiment, we could show that compound **16** was also active in the thioglycollate-induced peritonitis model in the mouse.[13,14] The maximal inhibition of neutrophil extravasation in this model was about 50% at a dose of 10 mg/kg sc.[15]

In summary, optimization of the lead compound **3** through a combined parallel synthetic and classical medicinal chemistry effort resulted in potent inhibitors of IKK2. On a cellular level we could show that these compounds inhibit the IκBα degradation and downstream events, like adhesion molecule expression. However, the selectivity of these compounds for the IKK pathway is only moderate in cellular assays. Compound **16** is orally bio-available in rats and mice, and we could demonstrate significant in vivo activity in an acute model of cytokine release. Future efforts will focus on further improvements in potency and selectivity for this compound series.

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8. IκBα degradation assay: for the assay, THP-1 cells were transferred to 96-well plates at a density of 600,000 cells per well and stimulated with 10 ng/ml TNFα for 12 min at rt. For measuring the inhibition of TNFα-stimulated IκBα degradation, the test compounds were dissolved in DMSO and added to the cell suspension prior to addition of TNFα. The concentration of IκBα in each sample was analyzed by Western blotting. Each compound was tested in duplicate and estimations of IC_{50} values were done based on densitometric scanning or visual inspection.
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10. Bach, E. A.; Aguet, M.; Schreiber, R. D. *Annu. Rev. Immunol.* **1997**, *15*, 563.
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12. This result correlates nicely with the ability of compound **16** to inhibit LPS induced TNFα release from hPBMC with an IC_{50} of 0.3 μM.
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15. Peritonitis was induced at $t = 0$ by ip injection of thioglycollate and allowed to develop for 4 h. The compound dissolved at 1 mg/ml D(+)glucose/PEG400/H_2O and then further diluted in PEG400/H_2O was applied sc at $t = 0$. Control animals received an injection of sterile saline only. After 4 h, the animals were sacrificed, PMNs migrated into peritoneum were counted and their number was compared to vehicle control.