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4-Anilino-6-phenyl-quinoline inhibitors of mitogen activated protein kinase-activated protein kinase 2 (MK2)

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ABSTRACT

A class of inhibitors of mitogen activated protein kinase-activated kinase 2 (MK2) was discovered via high-throughput screening. This compound class demonstrates activity against the enzyme with sub-μM IC_{50} values, and suppresses LPS-induced TNFα levels in THP-1 cells. MK2 inhibition kinetic measurements indicated mixed binding approaching non-ATP competitive inhibition.

Mitogen activated protein kinase-activated protein kinase 2 (MK2) is implicated in the regulation of synthesis of key pro-inflammatory cytokines such as TNFα and IL-6. By inhibiting MK2 therapeutic benefits are expected in a wide range of inflammatory diseases. MK2 is a distal kinase in the p38 MAPK signaling module where it is phosphorylated and activated preferentially by the p38 α and β isoforms. The role of the p38 pathway in inflammation is well established as defined by the anti-inflammatory effects of the p38αβ inhibitor SB203580 and related compounds.[1] MK2, being a more distal component of the p38 cascade, appears as a highly attractive target since it has been shown that MK2 is essential for LPS-induced biosynthesis of several pro-inflammatory cytokines in macrophages.[2]

Furthermore, it was recently shown that MK2 regulates biosynthesis of TNFα and IL-6 independently at different post-transcriptional levels. Whereas MK2 regulates biosynthesis of IL-6 at the level of mRNA stabilization, TNFα production is mainly controlled through translational control.[3] Using MK2 deficient mice, LPS-induced production of TNFα and NO were inhibited, and the phosphorylation of Hsp25/27 was severely affected.[4] These results suggest that MK2 may be a relevant target of the SAPK2 (stress-activated protein kinase 2) pathway in inflammation.

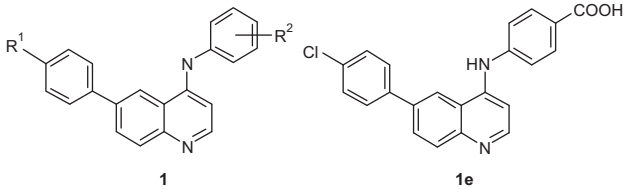
Recently, several reports of MK2 inhibitors have been published.[5]

From screening the AstraZeneca core compound collection we identified a novel series of 6-aryl-4-anilinoquinoline based inhibitors of MK2 exemplified by the compound **1e**, with an IC_{50} value of 12 μM (Fig. 1).[6]

To further explore the potential residing in this class of MK2 inhibitors we initiated a synthetic program to explore the structure–activity relationships. We measured inhibition constants to get kinetics related information.

The preparation of the 6-aryl-4-anilinoquinolines was readily accomplished starting from 6-bromo-4-chloroquinoline[7] according to Scheme 1.

The coupling reaction was performed using standard Suzuki conditions, adding the boronic acid in slight excess, with $Pd(dppf)_2Cl_2$ being the preferred catalyst. No coupling was observed in the quinoline 4-position, and yields were typically in the 70% range. The nucleophilic aromatic substitution in the 4-position proceeded smoothly using equimolar amounts of aniline in a sealed tube at 200 °C using *N*-methyl pyrrolidone (NMP) as solvent. Among the first set of compounds synthesized little improvement of potency was observed, Table 1. Most compounds showed an inhibition equal to or inferior to that observed for the initial hit compound **1e**. Inter-



1 1e

Figure 1. 6-Aryl-4-anilinoquinoline MK2 inhibitors.

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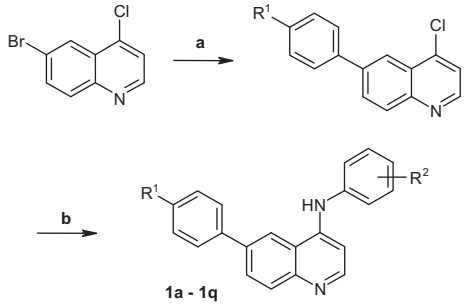
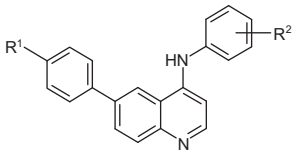


Scheme 1. Reagents and conditions: (a) $ArB(OH)_2$, $Pd(dppf)_2Cl_2$, Na_2CO_3, EtOH/toluene, 120 °C. (b) $ArNH_2$, NMP, 200 °C, sealed tube. Overall yields are ~70%.

Table 1
MK2 inhibition[6] for compounds **1a–q**



Compd	R^1	R^2	MK2 IC_{50} (μM)
1a	H	*p*-H	>200
1b	H	*p*-$(CH_2)_2OH$	22
1c	H	*p*-Et	41
1d	H	*p*-Morpholine	120
1e	Cl	*p*-COOH	12
1f	Cl	*p*-OH	23
1g	Cl	*p*-Morpholine	24
1h	Cl	*p*-$(CH_2)_2OH$	29
1i	Cl	*p*-CN	27
1j	Cl	*p*-CO-piperazine-4-Me	15
1k	CH_3	2-OH, 5-Cl	33
1l	CH_3	*p*-OH	25
1m	CH_3	*p*-CN	27
1n	CH_3	2-CH_3, 3-CH_3	35
1o	H	2-OH, 5-Cl	43
1p	Cl	2-F, 4-Cl	121
1q	H	*p*-(*N*-methyl piperazine)	5.2

estingly the addition of *p*-(*N*-methyl piperazine) substituted anilines provided a twofold improvement of the potency (compound **1q**).

Substituent containing *o*- and *m*-substituted anilines as well as bicyclical anilines, for example, indole, indane, benzodioxole, and benzofuranone showed significantly reduced capacity to inhibit MK2 activity.

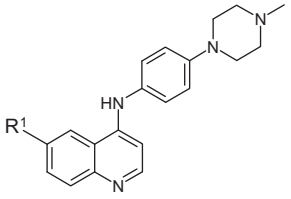
The improved potency observed for the compound **1q** prompted us to further investigate the *N*-methyl piperazine based sub-series. Indeed, expansion led to the identification of significantly improved MK2 inhibitors, Table 2. In this series *para*-substituted phenyl groups in the quinoline 6-position appear to provide the most potent inhibitors, with IC_{50}'s down to 0.4 μM (compound **2e**).

The *N*-methyl piperazine derivatives were prepared according to Scheme 2.

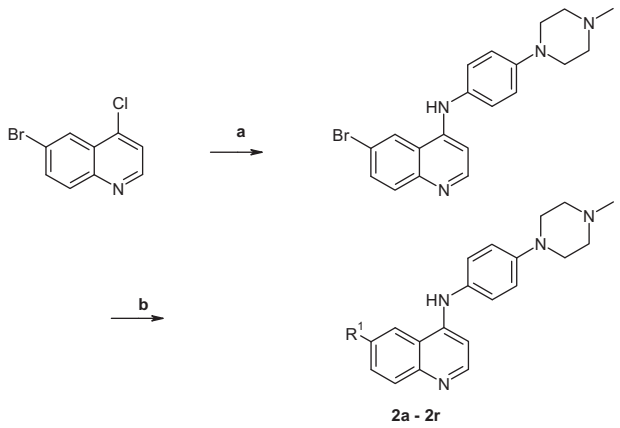
To maximize the overall yields an excess of boronic acid was used in the Suzuki couplings (Schemes 1a and 2b, respectively).

Aryl substitution on the quinoline 2-, 5-, 7- and 8-positions rendered less active compounds than the 6-substituted quinolines (**2**). The most potent compounds in this series, compounds **2e–2g** were also found to be active to suppress LPS-induced TNFα release from THP-1 cells,[8], for example, **2g** was found to have an EC_{50} of 4.3 μM (Fig. 2) which is almost identical to the value obtained for the other compounds. The lower activity in the cell based assay might be due

Table 2
MK2 IC_{50}'s for compounds **2a–r**



Compd	R^1	MK2 IC_{50} (μM)
2a	3-Thiophene	26
2b	H	>200
2c	Br	>200
2d	2-Thiophene	>200
2e	*p*-Br-phenyl	0.4
2f	*p*-Cl-phenyl	0.6
2g	*p*-F-phenyl	0.7
2h	*p*-COOH-phenyl	>200
2i	*m*-NO_2-phenyl	9.0
2j	*m*-NH_2-phenyl	14
2k	*m*-CF_3-phenyl	1.5
2l	*m*-Cl-phenyl	2.6
2m	*m*-CH_3O-phenyl	8.0
2n	*p*-CHO-phenyl	1.8
2o	*p*-CF_3-phenyl	1.4
2p	*p*-CH_3S-phenyl	0.7
2q	*p*-CH_3-phenyl	0.8
2r	*m*-CH_3-phenyl	1.4



Scheme 2. Reversed reaction order compared to Scheme 1. Reagents and conditions: (a) $ArNH_2$, NMP, 200 °C, sealed tube. (b) $ArB(OH)_2$, $Pd(dppf)_2Cl_2$, Na_2CO_3, EtOH/toluene, 120 °C. Overall yields are in the 50–65% range.

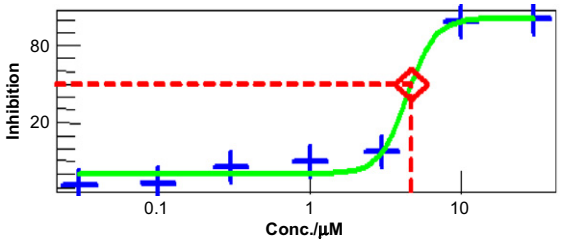


Figure 2. Inhibition of LPS-induced TNFα release from THP-1 cells by compound **2g**.

to bad permeability since compounds **2e–g, 2k**, **2p–r**, and **1q** were measured to have a P_{app} of 0.3 or less using a human Caco-2 permeability assay.

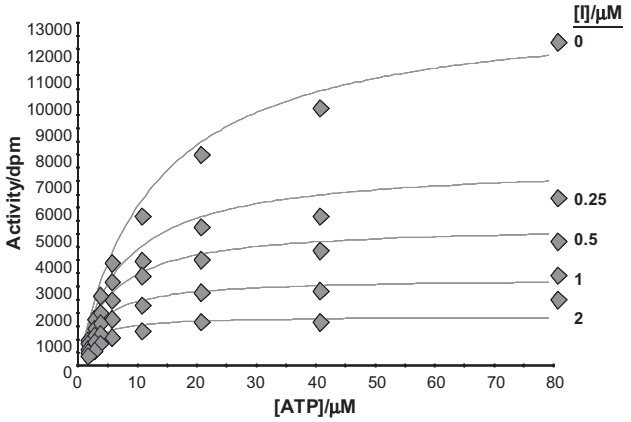


Figure 3. MK2 activity versus ATP concentration in the presence of 0, 0.25, 0.5, 1, and 2 μM of compound **2f** fitted with the equation for mixed inhibition using the EnzFitter software.

Table 3
Inhibition constants and goodness of fit obtained from the different models of inhibitor modality

	Mixed	Uncompetitive	Non-competitive	Competitive
K_m (μM)	12.6	13.3	8.80	7.21
K_{ic} (μM)	5.6		0.48	0.096
K_{iu} (μM)	0.32	0.31	0.48	
R^2	0.99	0.99	0.97	0.83

K_m, dissociation constant for the MK2-ATP complex; K_{ic}, competitive inhibition constant; K_{iu}, uncompetitive inhibition constant.[9]

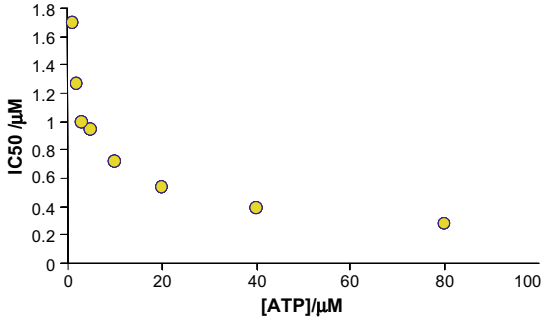


Figure 4. Inhibition of MK2 by **2f** at increasing ATP concentrations.

However, the indication is that this compound class may retain the activity also in a cell based context, in a manner consistent with MK2 enzyme inhibition.

To understand inhibition modality and to determine an inhibition constant, the plots of reaction velocity versus ATP concentration at various fixed inhibitor concentrations (**2f**) were fitted globally to the equations for competitive, non-competitive, and uncompetitive inhibition, respectively. This analysis strongly indicated a case of mixed inhibition with a dominating element of uncompetitive inhibition (Fig. 3).

Global analysis of the statistical parameters for goodness of fit confirmed that the models for mixed and uncompetitive inhibition best described the complete data set with better R^2 values than for competitive or non-competitive inhibition (Table 3). Using the model for mixed inhibition $K_{ic} \gg K_{iu}$, for example, the affinity for compound **2f** is almost 20-fold higher at the uncompetitive site than in the competitive (ATP-binding) site (Table 3).[9]

Compound **2f** inhibited MK2 more efficiently with increasing ATP concentrations (Fig. 4), further supporting the conclusion that it is mainly uncompetitive in nature.

In summary, we have reported on a set of small molecule MK2 enzyme inhibitors, also which may attenuate TNFα production in THP-1 cells. The measurement of inhibition kinetics of these inhibitors strongly indicates an uncompetitive binding mechanism. However, this class of amino-aryl-quinoline compounds were considered to be of less importance as starting points for lead optimization. Although many of the compounds are stable in the rat HW microsome metabolism assay (<10 μl/min/mg), less stability is usually found in human microsomes. Also this structural class shows a general cytotoxicity in human THP-1 cells using WST-1 dye and recording cell proliferation and viability, respectively (usually below 4 μM).

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6. MK2 activity (0.4 U/ml of activated MK2) was measured in 96-well micro titer plates in 8 mM MOPS, pH 7.0, 10 mM MgAc, 0.2 mM EDTA, 0.002% Brij 35, 1% glycerol, 0.04% BSA, 0.1% β-mercaptoethanol in the presence of 10 μM [^{33}P]-ATP (400 dpm/pmol) and 3 μM glycogen synthase peptide substrate (KKLNRTLSVA) in a total volume of 25 μl. After 30 min at room temperature reactions were quenched by the addition of 25 μl of 10% HAc, transferred to pre-wet Millipore P81 filter plates and incubated for 30 min at room temperature. Filters were washed with 5% Hac and counted (TriLux Microbeta) after adding 25 μl/filter of scintillation cocktail (OptiPhase SuperMix, Perkin Elmer).
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8. TNFα levels in THP-1 cell:
The assay was performed in a round bottom well format, where 100,000 THP-1 cells were incubated with or without compound for 45 min followed by a 5 h stimulation with 10 ng/ml of LPS at 37 °C. Cell-free supernatants were then collected and stored at −80 °C until use. TNFα concentrations in the supernatants were analyzed using a TNFα ELISA kit from R&D Systems according to the instructions of the manufacturer.
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