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In vivo and in vitro SAR of tetracyclic MAPKAP-K2 (MK2) inhibitors. Part I

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ABSTRACT

Pyrrolo[2,3-*f*]isoquinoline based amino acids, tetracyclic lactams and cyclic ketone analogues are described as novel MK2 inhibitors with IC_{50} as low as 5 nM and good selectivity profiles against a number of related kinases including ERK, p38α and JNKs. TNFα release was suppressed from human peripheral blood mononuclear cells (hPBMCs), and a representative compound inhibited LPS induced TNFα release in mice illustrating the potential of this series to provide orally active MK2 inhibitors.

Mitogen-activated protein kinases (MAPKs) belong to the Ser/Thr kinase family[1], control cytoskeletal architecture, cell-cycle progression and are implicated in inflammation and cancer.[2] The p38/MAPKAP kinase-2 (MAPKAP-K2; MK2) cascade plays a pivotal role in the production of proinflammatory cytokines, such as TNFα, IL-6 and IFNγ.[3] Moreover, MK2 knock-out mice are resistant to developing disease in arthritis models.[4] Thus, MK2 has emerged as a highly desirable target in the search for efficacious and safe anti-inflammatory drugs. Several reports on MK2 inhibitors from a variety of structural classes were published[5] including pyrrolopyrimidinones[6] and aminopyrazoles.[7] Recently the difficulties in developing MK2 inhibitors have been reviewed.[8]

Our search for MK2 inhibitors began with high throughput screening that identified the pyrrolo[2,3-*f*]isoquinoline amide **1** as a moderate MK2 inhibitor with an IC_{50} value of 3.8 μM (Fig. 1). **1** demonstrated structural similarities to the recently disclosed MK2 inhibitors **2**[5f] with a pyrrolopyridine scaffold and submicromolar IC_{50}. A chemistry program was initiated aimed at enhancing potency of MK2 inhibitors by combining structural features of **1** and **2**. Described herein are the resulting novel MK2 inhibitors **5–8**, **10**, **11**, **13**, **14** and their SAR.

Bromide **4**—the precursor of MK2 inhibitors **5–8**—was prepared from 3-chloro-5-aminoisoquinoline (Scheme 1), which was condensed with 3-oxobutyric acid *tert*-butyl ester to an enamine that cyclised under oxidative $Pd(OAc)_2/Cu(OAc)_2$ catalysis[9] to provide pyrroloisoquinoline **3**. BOC-protection followed by NBS treatment gave **4**, which was reacted with methylamine, ammonia, methylhydrazine and *tert*-butyl *N*-hydroxycarbamate. Suzuki[10] coupling and deprotection yielded amino acids **5A**, **6A** and the amino acid precursor of **7A**, which was cyclised with EDCI. Suzuki coupling with **8** (R^1 = Cl) was successful and yielded **8E**, while **7** (R^1 = Cl) did not survive the coupling conditions.

Boronic acids or their pinacol esters employed to introduce side chains R^1 = **A–C** and **E–K** were either commercially available or prepared as described previously.[11]

Amino acid **5A** was a potent and rather selective MK2 inhibitor (IC_{50} = 0.014 μM; Table 1), showing cellular efficacy (IC_{50} = 0.735 μM). Potential prodrugs of **5A**, such as the methyl ester and the primary amide (not shown in Table 1) were considerably weaker (ester: 11 μM; amide: 1.2 μM). The high potency of **5A** may be explained by possible salt formation with Lys93 and Asp207 of MK2 (see X-ray analysis, Figure 2). The *N*-methyl analogue **6A** led to a drop in MK2 inhibitory potency and cellular activity. Lactams **7A** and **8E** were both potent MK2 inhibitors with **7A** also inhibiting LPS induced TNFα release from hPBMCs (IC_{50} = 0.046 μM). The latter effect may partly be due to off-target kinase inhibition, since **7A** inhibited 6 out of 26 kinases[12] with

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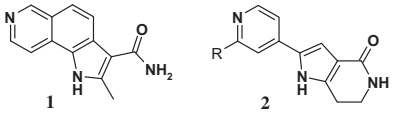
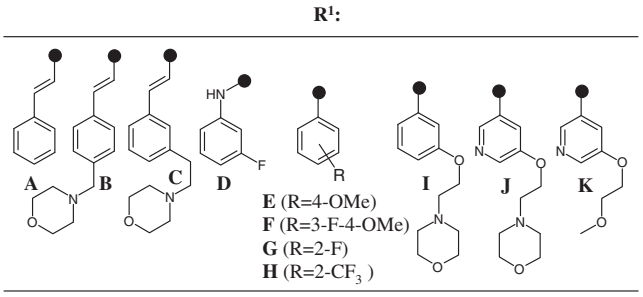


Figure 1. MK2 inhibitors. Compound **1**: HTS-hit. Compound **2**: Pfizer's MK2 inhibitors.[5f]

IC_{50} <1 μM. Since JNK2-inhibition may contribute to the inhibition of TNFα[13] release and toxicity, MK2 inhibitors with minimal JNK2-inhibition are desirable. **8E** showed a 23.7-fold preference for MK2 over JNK2 (Table 1), inhibited hsp27 phosphorylation with IC_{50} = 0.87 μM and LPS induced TNFα release with IC_{50} = 2.36 μM, which supports the notion, that its cellular efficacy was MK2-driven.

Tetracyclic MK2 inhibitors **10** and **11** with a 5-membered lactam-ring were prepared according to Scheme 2 from **4**, which was reacted with $MeNH_2$ or NH_3. The resulting amines were deprotected to amino acids and then cyclised to **9** with EDCI**.** Suzuki coupling with R^1–$B(OH)_2$ or its pinacol ester provided **10A–C**, **10E–K** and **11A**. 3-Fluoroaniline and **9** delivered **10D** via Buchwald[14] reaction conditions. Styrene derivatives **10A–C** (Table 2) were potent MK2 inhibitors (IC_{50} ⩽0.010 μM), but highly unselective. JNK2 (IC_{50} ⩽0.034 μM) by **10A–C** possibly contributed to the potent inhibition of LPS induced TNFα in hPBMCs (IC_{50} ⩽0.014 μM)**.** Aniline derivative **10D** had a 75-fold higher affinity for JNK2 (IC_{50} = 0.001 μM) than for MK2. Compound **10H** among the phenyl derivatives **10E–I** demonstrates the importance of coplanarity between the tetracyclic core and the R^1-substituent. The large 2-trifluoromethyl group in **10H** forces the phenyl group out of plane and leads to a weak MK2 inhibition (IC_{50} = 0.9 μM), while the small 2-fluorophenyl group of **10G** stays nearly in plane with the tetracycle and remains a potent MK2 inhibitor (IC_{50} = 0.085 μM). Pyridyl derivatives **10J** and **10K** were the most potent MK2 inhibitors (IC_{50} = 0.005 μM) of the above series with a still modest 15- and sevenfold selectivity over JNK2. Consequently, their cellular efficacy may not be exclusively MK2-driven. N-Methylation of the lactam—exemplified by **11A**—was not tolerated. This is in agreement with the finding, that the lactam-NH binds to Asp207 (see X-ray analysis, Fig. 2).

Tetracycles **13** with a six-membered lactam-ring were prepared from 3-chloro-5-aminoisoquinoline and cyclopentane-1,3-dione, which were condensed to an enaminone intermediate and then



Scheme 1b. R^1-substituents.

Table 1

Compd	MK2[a] (μM)	TNF[b] (μM)	*p*-hsp27[c] (μM)	JNK2[d] (μM)	Selectivity[d]
5A	0.014	0.735	nt	nt	2
6A	0.060	1.240	nt	nt	nt
7A	0.025	0.046	nt	nt	6
8E	0.023	2.359	0.875	0.547	2

IC_{50} values are reported as the mean of ⩾2 experiments with a standard deviation of less than ±50%.

[a] MK2 enzyme assay is performed as described.[7]

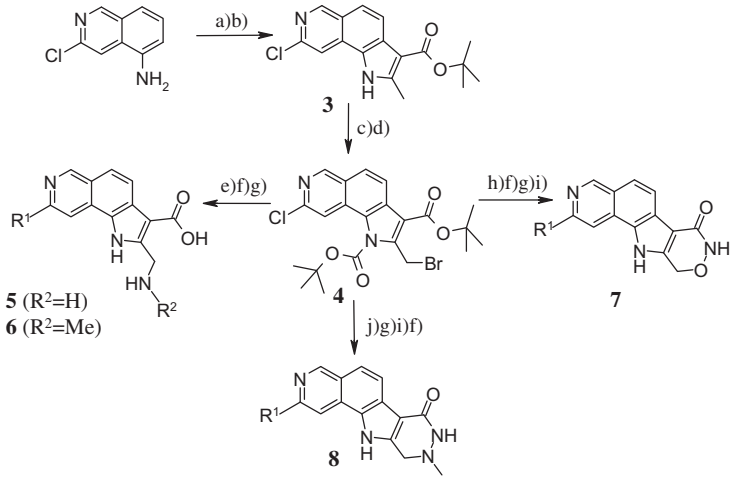
[b] Inhibition of LPS stimulated release of TNFα from hPBMC is performed as described.[7]

[c] hsp27 phosphorylation is performed as described.[7]

[d] Number of kinases inhibited with IC_{50} <1 μM out of a panel of 26 kinases.[12] nt: not tested. R^1-substituents **A**–**H** are defined in Scheme 1b.

oxidatively cyclised with $Pd(OAc)_2/Cu(OAc)_2$ to render ketone **12** (Scheme 3). The oxime derivative of **12** underwent a Beckmann[15] rearrangement to deliver **13** (R^1 = Cl). Suzuki couplings led to the styrene derivatives **13B** and **13C**, the phenyl analogues **13F** and **13I** and the pyridyl substituted tetracycles **13J** and **13K. 14F**—a tetracycle with an annulated cyclopentanone—was obtained from **12** via Suzuki coupling.

The styrene derivatives **13B** and **13C** (Table 3) were potent MK2 inhibitors (IC_{50} ⩽0.005 μM), but unselective as expected. The phenyl derivatives **13F** and **13I** were potent MK2 inhibitors (IC_{50} ⩽0.016 μM) and displayed good selectivity. Compound **13F** showed a 100-fold selectivity over JNK2, potently inhibited hsp27 phosphorylation (IC_{50} = 0.5 μM) and the LPS induced release



Scheme 1a. Reagents and conditions: (a) 3-oxobutyric acid *tert*-butyl ester, HOAc, 50 °C, 4 h, 66%; (b) $Pd(OAc)_2$, $Cu(OAc)_2$, DMF, 120 °C, 10 min, 63%; (c) BOC_2O, DMAP, diethylene glycol dimethylether, 120 °C, 30 min, 97%; (d) NBS, dibenzoyl peroxide, CCl_4, reflux 2.5 h, 91%; (e) dioxane, $MeNH_2$ or NH_{3concd} microwave, 100 °C, 15 min, 60–70%; (f) R^1–$B(OH)_2$ or pinacol ester, $PdCl_2$(dppf), K_2CO_3, DMF/H_2O, 90 °C, 1.5 h, 40–45%; (g) HCl_{concd} 2 min, 25 °C; (h) *tert*-butyl *N*-hydroxycarbamate, K_2CO_3, dioxane, reflux 15 min., 65%; (i) EDCI, HOBt, DMF, 25 °C, 4.5 h, 55%; (j) methylhydrazine, $NaHCO_3$, dioxane, reflux 3 h.

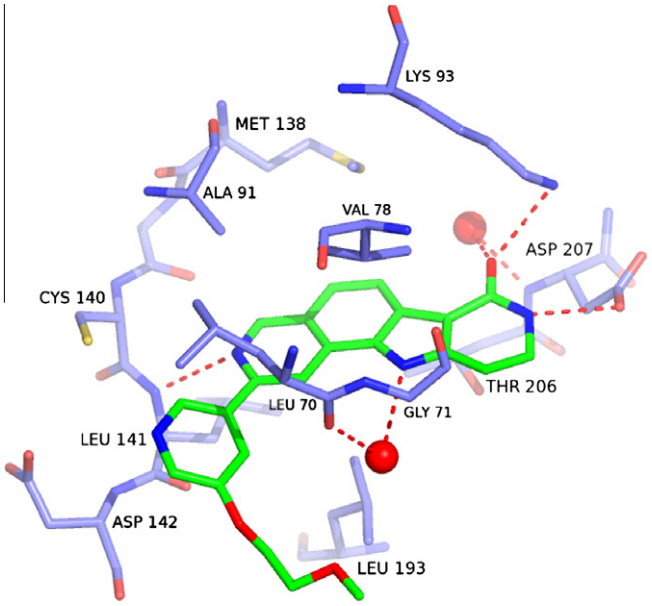
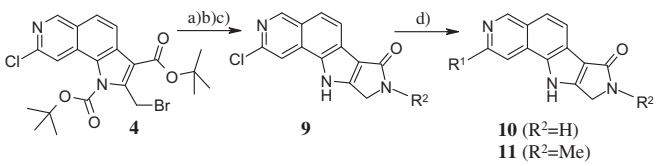


Figure 2. Crystal structure of **13K** bound to MK2.[16]



Scheme 2. Reagents and conditions: (a) dioxane, $MeNH_2$ or NH_{3concd} microwave, 100 °C, 15 min, 60–70%; (b) HCl_{concd} 2 min, 25 °C; (c) EDCI, HOBt, DMF, 25 °C; (d) R^1–$B(OH)_2$ or pinacol ester, $PdCl_2$(dppf), K_2CO_3, DMF/H_2O, 90 °C, 1.5 h, 40–45%. For **10D**: 3-fluoroaniline, $PdCl_2$(dppf), R(+)-BINAP, 2-dicyclohexyl-phosphino-2′-(*N*,*N*-dimethylamino)-biphenyl, Cs_2CO_3, DMF, microwave, 160 °C, 4 h, 34%. R^1-substituents **A**–**H** are defined in Scheme 1b.

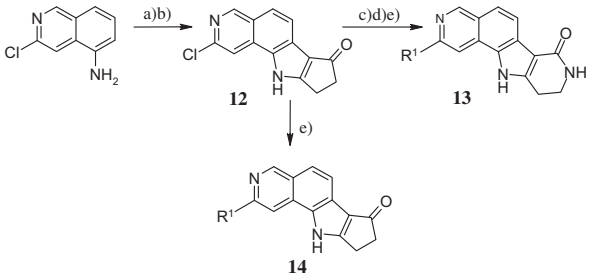
of TNFα (IC_{50} = 0.197 μM) from hPBMCs. Ketone **14F** was ~10-fold less potent against MK2 (IC_{50} = 0.16 μM) and similarly selective. Pyridyl substituted tetracycles **13J** and **13K** were potent MK2 inhibitors (IC_{50} ⩽0.012 μM). **13K** showed a 54-fold preference for MK2 over JNK2, inhibited hsp27 phosphorylation (IC_{50} = 0.7 μM) and LPS induced TNFα release (IC_{50} = 0.26 μM). The cellular efficacies of both **13F** and **13K** are therefore linked with high probability to MK2 inhibition.

Tetracycles **16** with a seven-membered lactam-ring were prepared from 3-chloro-5-aminoisoquinoline and cyclohexane-1,3-dione in analogy to the tetracycles with a six-membered lactam-ring described above (Scheme 4).

Table 2

Compd	MK2[a] (μM)	TNF[b] (μM)	*p*-hsp27[c] (μM)	JNK2[d] (μM)	Selectivity[d]
10A	0.010	0.014	nt	0.002	9
10B	0.008	0.005	nt	0.022	19
10C	0.010	0.014	nt	0.034	15
10D	0.073	0.015	nt	0.001	12
10E	0.027	0.198	nt	nt	nt
10G	0.085	0.912	5.078	0.030	nt
10H	0.900	nt	nt	nt	nt
10I	0.032	0.280	1.28	0.011	6
10J	0.005	0.556	9.33	0.075	1
10K	0.005	0.416	1.68	0.038	3
11A	1.189	nt	nt	nt	nt

IC_{50} values are reported as the mean of ⩾2 experiments with a standard deviation of less than ±50%.
[a-d] See Table 1.



Scheme 3. Reagents and conditions: (a) 3-chloro-5-aminoisoquinoline and cyclopentane-1,3-dione heated as melt at 120 °C for 25 min; (b) $Pd(OAc)_2$, $Cu(OAc)_2$, DMF, 120 °C, 25 min., 88%; (c) NH_2OH·HCl, pyridine, reflux 3 h, 70%. d) PPA, 130 °C, 75 min, 70% (e) R^1–$B(OH)_2$ or pinacol ester $PdCl_2(PPh_3)_2$, 2 N Na_2CO_3, 1-propanol, microwave, 160 °C, 15 min., 25–56%. R^1-substituents **A**–**H** are defined in Scheme 1b.

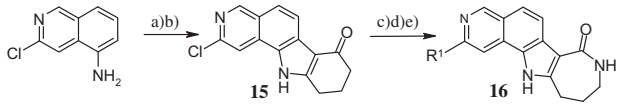
Table 3

Compd	MK2[a] (μM)	TNF[b] (μM)	*p*-hsp27[c] (μM)	JNK2[d] (μM)	Selectivity[d]
13B	0.001	0.009	nt	0.198	13
13C	0.005	0.032	nt	0.076	8
13F	0.015	0.097	0.5	1.42	0
13I	0.016	0.294	1.3	0.269	3
13J	0.010	0.283	2.5	0.418	2
13K	0.012	0.260	0.7	0.649	2
14F	0.160	1.400	5.8	2.200	0

IC_{50} values are reported as the mean of ⩾2 experiments with a standard deviation of less than ±50%.
[a-d] see Table 1.

Styrene substituted tetracycles **16A–C** (Table 4) were weak MK2 inhibitors (IC_{50} ⩽0.253 μM) and unselective as expected. Phenyl substituted tetracycles **16E** and **16I** were not much weaker MK2 inhibitors than their five- and six-membered lactam analogues, but displayed weak cellular activity. Pyridyl analogues **16J** and **16K** were ~8-fold weaker against MK2 than six-membered lactam analogues **13J**, **13K** and showed almost no cellular activity.

The lactam-ring size of the tetracycles determines their relative potency at MK2, with the six-membered lactams being more potent than the five-membered lactams, the latter being more potent than the seven-membered lactams. R1-substituents had a



Scheme 4. Reagents and conditions: (a) 3-chloro-5-aminoisoquinoline and cyclohexane-1,3-dione heated as melt at 120 °C for 25 min; (b) $Pd(OAc)_2$, $Cu(OAc)_2$, DMF, 120 °C, 25 min, 37%; (c) NH_2OH·HCl, pyridine, reflux 3 h, 70%; (d) PPA, 130 °C, 75 min., 70%; (e) R^1–$B(OH)_2$ or pinacol ester $PdCl_2(PPh_3)_2$, 2 N Na_2CO_3, 1-propanol, microwave, 160 °C, 15 min., 29–63%. R^1-substituents **A**–**H** are defined in Scheme 1b.

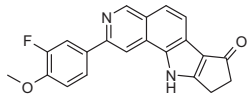
Table 4

Compd	MK2[a] (μM)	TNF[b] (μM)	p-hsp27[c] (μM)	JNK2[d] (μM)	Selectivity[d]
16A	0.116	0.202	nt	0.085	8
16B	0.253	0.140	nt	0.980	4
16C	0.172	>1	nt	0.700	5
16E	0.072	0.580	nt	nt	1
16I	0.167	>3	nt	nt	0
16J	0.080	8.6	>30	3.170	0
16K	0.096	8.38	8.3	2.700	0

IC_{50} values are reported as the mean of ⩾2 experiments with a standard deviation of less than ±50%.
[a-d] See Table 1.

modulating effect on potency and selectivity. High selectivity was conferred by phenyl and pyridyl substituents, while styryl substituents and anilines showed affinity to off-target kinases such as JNK2. Insight into the binding mode of tetracycles was obtained by X-ray crystallography of **13K** with MK2 (47–364). **13K** bound to the hinge region with a classical H-bond formed between the N-atom of its pyridine and the backbone amide nitrogen of Leu141. The pyrrole NH of **13K** bound to a water molecule, which was H-bonding to the backbone carbonyl of Leu70. The lactam carbonyl showed H-bonding to Lys93, the lactam N–H bound to Asp207 (Fig. 2).

Several tetracyclic lactams were tested[17] in vivo, but none showed oral efficacy. The tetracyclic ketone **14F** however proved to be orally bioavailable and inhibited 73% of LPS induced TNFα release in mice with plasma levels reaching 13 μM after 2 h.[17] Since **14F** was shown to inhibit LPS induced release of TNFα from hPBMCs (Table 3) and hsp27 phosphorylation with IC_{50} values of 1.4 μM and 5.8 μM respectively, one may assume, that this in vivo effect is MK2-driven.



14F

Further optimization of these compound classes for oral bioavailability is ongoing.

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16. The X-ray coordinates are deposited with RCSB Protein Data Bank, deposition code is 3M42. The protein used in this study is a segment of MK2 containing residues 47–364δ(216–237)G.
17. MK2 inhibitors (100 mg/kg po) were administered to OFI mice (female, 8 weeks old), followed by LPS injection (20 mg/kg) 1 h later. One hour post LPS injection the experiment was terminated and blood withdrawn. Compound blood levels were determined by LC–MS/MS and plasma levels of mouse TNFα determined by ELISA.