

## Modulating ADME Properties by Fluorination: MK2 Inhibitors with Improved Oral Exposure

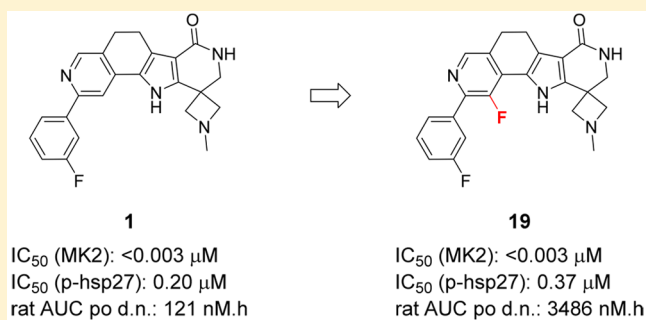
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### Supporting Information

**ABSTRACT:** MAP-activated protein kinase 2 (MK2) plays an important role in the regulation of innate immune response as well as in cell survival upon DNA damage. Despite its potential for the treatment of inflammation and cancer, to date no MK2 low molecular weight inhibitors have reached the clinic, mainly due to inadequate absorption, distribution, metabolism, and excretion (ADME) properties. We describe here an approach based on specifically placed fluorine within a recently described pyrrole-based MK2 inhibitor scaffold for manipulation of its physicochemical and ADME properties. While preserving target potency, the novel fluoro-derivatives showed greatly improved permeability as well as enhanced solubility and reduced *in vivo* clearance leading to significantly increased oral exposure.

**KEYWORDS:** MK2, fluorine, permeability, ADME properties, pharmacokinetic, directed ortho metalation



Tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) is a key pro-inflammatory cytokine and the major driver of systemic inflammation.<sup>1,2</sup> Its overexpression is linked to several autoimmune diseases including rheumatoid arthritis, psoriasis, and inflammatory bowel diseases. At present, patients can profit from *anti*-TNF $\alpha$  therapy using marketed biologics, like adalimumab (Humira), etanercept (Enbrel), or infliximab (Remicade).<sup>3</sup> Nonetheless, a small molecular weight orally bioavailable drug blocking TNF $\alpha$  production would be highly desirable. MK2, or MAPKAPK2 (mitogen-activated protein kinase activated protein kinase 2), is a serine/threonine kinase and a downstream substrate of p38.<sup>4–8</sup> Due to its important role in TNF $\alpha$  secretion and inflammation, p38 was extensively explored as a therapeutic target for the treatment of autoimmune diseases.<sup>9</sup> Despite several p38 inhibitors entering clinical trials,<sup>10</sup> none of them reached phase 3, mainly due to their poor safety profile and observed hepatotoxicity and cardiotoxicity (p38 is involved in the regulation of more than 60 substrates), but also their lack of long-term efficacy due to counter activation of transforming growth factor  $\beta$ -activated kinase 1 (TAK1) as a result of p38 inhibition (TAK1 feedback loop).<sup>11</sup> Hence, MK2 was proposed as an alternative target for inhibition of the pathway while avoiding p38-dependent side effects.<sup>7</sup> Beyond its role in the regulation of inflammation, MK2 was recently shown to be involved in the G2/M checkpoint arrest upon DNA damage, thus contributing to the resistance of p53-deficient tumors to cisplatin.<sup>12</sup> In addition, MK2 also displayed a synergistic effect with checkpoint kinase 1 (Chk1) inhibition leading to a mitotic catastrophe in KRAS mutant cells.<sup>13</sup> Most of the low molecular weight MK2 inhibitors reported to date are restricted by their

physicochemical properties leading to mediocre cellular potency and/or efficacy in animal models,<sup>7,8</sup> ultimately preventing them from advancing into clinical trials.

Oral bioavailability, the fraction of the oral dose reaching systemic circulation, is predominantly dependent on intestinal absorption of a drug and its first-pass metabolism.<sup>14,15</sup> Oral absorption relies on the drug's permeability and aqueous solubility and is influenced by four key-parameters: molecular weight, logP, number of hydrogen bond donors (HBD) and acceptors (HBA), and form the basis of Lipinski's "rule of five" for prediction of absorption.<sup>16</sup> As a part of our interest in MK2 inhibitors,<sup>17–21</sup> we have recently described a series of pentacyclic type 1 (ATP-competitive) MK2 inhibitors<sup>20,21</sup> represented by compound **1** (Table 1). During lead optimization, combining good cellular potency with acceptable oral exposure in rodents was challenging in this series. Usually, potent MK2 inhibitors like **1** suffered from insufficient oral bioavailability and permeability while not violating the rule of five. The primary cause of the unfavorable oral exposure appeared to be the presence of a pyrrole-NH. As illustrated by compound **2** (Table 1), methylation of the pyrrole-NH greatly improved permeability, as assessed by parallel artificial membrane permeability assay (PAMPA). Notably, the superior penetration of derivative **2** did not result from a presumably increased lipophilicity as the logD<sub>7.4</sub> measured for compounds **1** and **2** was virtually the same (Table 1). This indicates that

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Table 1. MK2 Inhibitors: Challenge to Combine Cellular Potency with Good Oral Exposure

Compd.	Structure	MK2 <sup>a</sup> [ $\mu$ M]	p-hsp27 <sup>b</sup> [ $\mu$ M]	TNF $\alpha$ <sup>c</sup> [ $\mu$ M]	logPAMPA <sup>d</sup> [cm/s]	logD <sub>7.4</sub>	BAV <sup>e</sup> [%]	AUC po dn <sup>f</sup> [nmol.h/L]	CL <sup>g</sup> [mL/min/kg]
1		<0.003	0.20	0.17	-5.2	3.4	27 $\pm$ 8	121 $\pm$ 35	100 $\pm$ 29
2		0.007	2.33	1.54	-3.9	3.7	53 $\pm$ 24*	2415 $\pm$ 1032*	10 $\pm$ 3*
3		0.006	4.51	0.45	-4.6	3.0	151 $\pm$ 58	2154 $\pm$ 833	30 $\pm$ 3

IC<sub>50</sub> values determined as a mean ( $n \geq 2$ ) of "human MK2 kinase activity," inhibition of hsp27 phosphorylation in anisomycin stimulated THP-1 cells, and LPS stimulated TNF $\alpha$  release from human PBMCs. <sup>d</sup>Permeability determined by high-throughput PAMPA. <sup>e</sup>Oral bioavailability  $\pm$  SD calculated as dose normalized ratio of extravascular AUC<sub>extrap</sub> to iv AUC<sub>extrap</sub>; both parameters determined as a mean of 4 animals (female Sprague–Dawley rat). <sup>f</sup>Exposure (AUC; dn = dose-normalized to 1 mg/kg)  $\pm$  SD measured as a mean of 4 animals (female Sprague–Dawley rat) after po dosing (3 mg/kg) using CMC/water/Tween (0.5:99:0.5) formulation. <sup>g</sup>Clearance measured as a mean  $\pm$  SD of 4 animals (Sprague–Dawley rat) after iv dosing (1 mg/kg) using NMP/PEG200 (30:70) formulation. \*Values determined over 8 h (= t-last).

HBD strength of the pyrrole-NH rather than lipophilicity<sup>22</sup> might be responsible for the poor permeability of such compounds, a hypothesis further supported by analog 3. Counterintuitively, an additional HBA in 3 led to improved permeability suggesting the pyrimidine nitrogen to influence the pyrrole-NH HBD strength. The overproportional increase in rat oral exposure observed for compounds 2 and 3 could be explained by further improvement in their rat *in vivo* clearance compared to 1 (Table 1).

Unfortunately, the described modifications of derivatives 2 and 3 reduced their potency on MK2 biochemical activity and in cellular systems (inhibition of heat shock protein 27 (hsp27) phosphorylation in anisomycin stimulated human acute monocytic leukemia cell line (THP-1) or by inhibition of TNF $\alpha$  release in LPS stimulated human peripheral blood mononuclear cells (PBMCs)). Whereas this was not surprising for analog 2, since the pyrrole-NH was shown to be involved in a water mediated interaction with the enzyme,<sup>19,20</sup> the decreased MK2 potency for pyrimidine derivative 3 was less predictable. This might, however, be an indication again for the reduction in pyrrole-HBD strength caused by the additional nitrogen in 3.

A suitably positioned fluorine<sup>23</sup> has been shown to improve permeability and oral bioavailability of amides with otherwise poor oral absorption while retaining the target potency as exemplified by paired derivatives 4/5<sup>24</sup> and 6/7<sup>25</sup> (Figure 1). Since a C–F group can serve as a bioisostere of nitrogen in azines and azoles,<sup>26–28</sup> we wished to explore the effect of fluorine in the 3-position of pyridine (like in 19, Figure 2) for its ability to modulate permeability and oral absorption while hoping to keep the MK2 potency.

In analogy to the previously described synthesis of compound 1,<sup>20,21</sup> the synthesis of the proposed F-pentacycle 19 was envisioned to be achieved by the Hantzsch pyrrole synthesis using bromoketone 8 and spiroperidinedione 9 (Figure 2). While compound 9 was prepared according to the described procedure,<sup>21</sup> bromoketone 8 could be obtained from

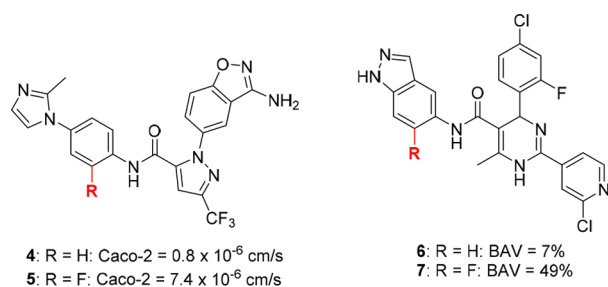


Figure 1. Examples of a positive influence of fluorine on permeability and bioavailability.

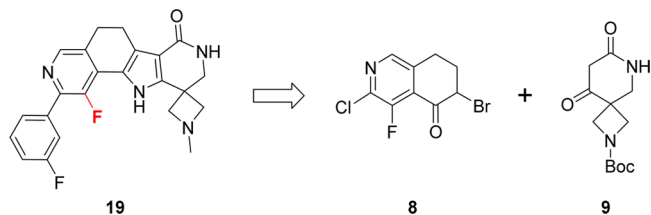
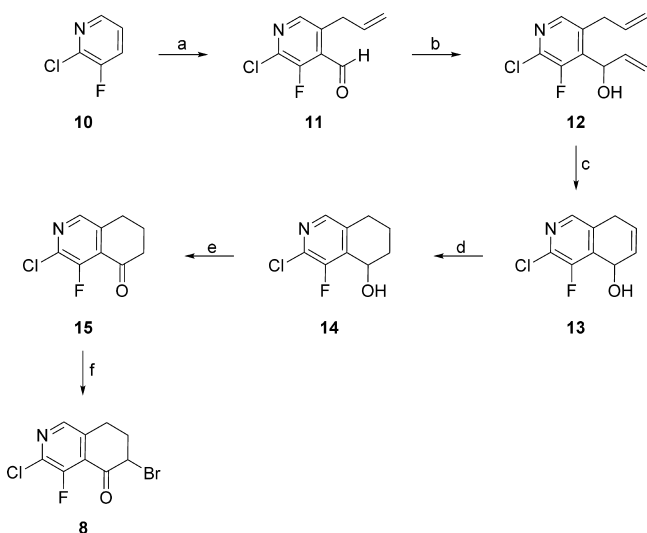


Figure 2. Proposed fluoro-containing analog 19 and its synthesis plan.

compound 15 (Scheme 1). Synthesis of 15 has been recently developed and published by our group<sup>29</sup> and starts with the preparation of tetrasubstituted pyridine 11 from commercially available 3-fluoro-2-chloropyridine (10) using Comins' protocol<sup>30</sup> for sequential directed *ortho*-metalations (DOM). The first DOM of 10 was achieved with *t*-BuLi, and the formed 4-pyridyl anion readily underwent the Bouveault reaction with *N*-formyl-*N,N,N'*-trimethylethylene-1,2-diamine providing an  $\alpha$ -amino alkoxide. This protected aldehyde intermediate allowed for a second DOM with *n*-BuLi leading to a 5-pyridyl lithium species that had to be transmetalated into a cuprate in order to enable its alkylation by allyl bromide. The five-step one-pot reaction provided the desired product 11 in a satisfactory yield. The required bromoketone 8 was obtained from 11 by addition

**Scheme 1. Synthesis of Fluoropyridine Building Block 8 Using Comins' Method for Double Directed *ortho*-Metalation as a Key Step<sup>a</sup>**



<sup>a</sup>Reagents and conditions: (a) (1) *t*-BuLi, THF,  $-78$  °C, 1 h, (2)  $\text{Me}_2\text{NCH}_2\text{CH}_2\text{NMeCHO}$ ,  $-78$  to  $-40$  °C, (3) *n*-BuLi,  $-40$  to  $-30$  °C, 3 h, (4) CuBr,  $-30$  to  $0$  °C, 1 h, (5) allyl bromide,  $-30$  to  $-10$  °C, 1 h (41%); (b) vinyl bromide, THF,  $0$  °C, 1 h (76%); (c) Grubbs II (*cat.*),  $\text{CH}_2\text{Cl}_2$ , rt, 1 h (97%); (d)  $\text{H}_2$  (1 atm), PtO<sub>2</sub> (*cat.*), MeOH, rt, 1 h (73%); (e)  $(\text{ClCO})_2$ , DMSO, Et<sub>3</sub>N,  $\text{CH}_2\text{Cl}_2$ ,  $-60$  °C to rt, 4 h (89%); (f) Br<sub>2</sub>, HBr, AcOH, rt to  $35$  °C, 15 min (98%).

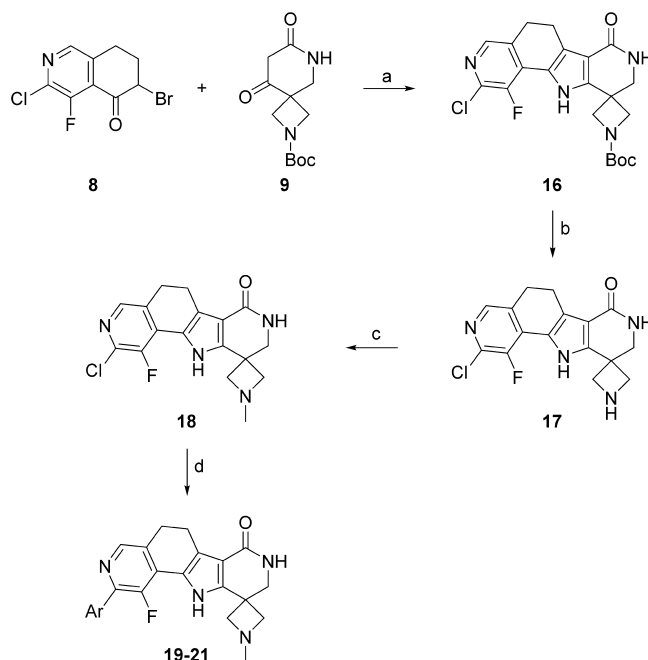
of vinyl Grignard to the aldehyde, followed by ring-closing metathesis of diene **12**, reduction of alkene double bond in **13**, oxidation of alcohol **14**, and  $\alpha$ -bromination of the formed ketone **15** (Scheme 1).

Condensation of the bromoketone **8** with the spiro-piperidinedione **9** in the presence of ammonium acetate provided the expected pyrrole intermediate **16** (Scheme 2). After removal of the Boc-protecting group, the free azetidine-NH in **17** was methylated by formaldehyde under reductive amination conditions. The final compounds **19–21** were obtained from the chloropyridine intermediate **18** by Suzuki coupling.

Gratifyingly, the newly obtained 3-fluoropyridine containing compounds **19–21** showed robust MK2 inhibition (Table 2) and maintained potency at the level of non-fluoro analog **1** as assessed by biochemical and cellular assays (p-hsp27 and TNF $\alpha$ ). Furthermore, these new derivatives potently inhibited TNF $\alpha$  release from human blood, with compound **21** being identified as the best candidate. In line with our hypothesis, the permeability of the fluoro-containing derivatives could indeed be significantly improved (by 1 log unit in PAMPA comparing analog **19** to **1**). Similarly to *N*-methylated pyrrole analog **2**, the improved permeability of fluoro-analog **19** was not driven by an increased lipophilicity as its logD<sub>7.4</sub> remained comparable to that of compound **1** (Table 2).

In addition to the superior permeability, fluorination of the pentacyclic core led also to improved solubility<sup>31</sup> (0.032 g/L for **19** vs 0.004 g/L for **1**) as well as rat *in vivo* clearance (11 mL/min/kg for **19** vs 100 mL/min/kg for **1**). It is quite remarkable what effect a single atom can have<sup>32</sup> on multiple ADME parameters, especially considering that permeability typically diverges from solubility and clearance during optimization. The improved physicochemical properties consequently led to

**Scheme 2. Synthesis of Fluoro-Containing Analogs 19–21<sup>a</sup>**

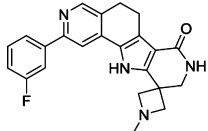
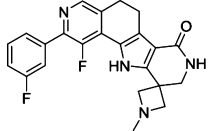
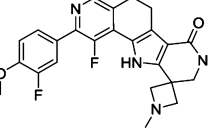
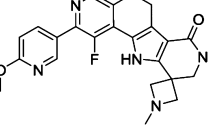


<sup>a</sup>Reagents and conditions: (a)  $\text{NH}_4\text{OAc}$ , MeOH,  $60$  °C, 3 h (85%); (b) HCl, dioxane, rt, 3 h (100%); (c) formaldehyde,  $\text{NaBH}(\text{OAc})_3$ , DIPEA,  $\text{CH}_2\text{Cl}_2$ , rt, 16 h (39%); (d) Ar-B(OH)<sub>2</sub>, Na<sub>2</sub>CO<sub>3</sub>, PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>, PPh<sub>3</sub>, *n*-PrOH/H<sub>2</sub>O,  $150$  °C, 15 min (53–66%).

better oral absorption of such compounds as revealed by an increase in rat oral bioavailability (Table 2). The enhanced oral absorption together with decreased clearance observed for the fluoro-containing analogs resulted in a significantly improved oral exposure (3486 nM·h for **19** vs 121 nM·h for **1**). Taken together with the attractive MK2 potency achieved with the fluoro analogs **19–21**, this study demonstrates that a fluorine atom can be a highly useful tool for tuning ADME properties while not interfering with target potency. This new modification may also enable further optimization of the scaffold toward MK2 inhibitors with improved *in vivo* efficacy.

In conclusion, a new fluoro-containing MK2 inhibitor scaffold has been described. After observation that the pyrrole-NH within the previously described pyrrole-based MK2 inhibitors was responsible for their poor physicochemical and ADME properties, an influence of a fluorine atom placed into its proximity was studied for the modulation of such parameters. The designed fluoro-containing analogs were synthesized using a Hantzsch pyrrole synthesis. The required fluoro-containing building block **8** could be assembled utilizing a remarkable one-pot, five-step sequence employing Comins' protocol for two subsequent directed *ortho*-metalations. The obtained fluoro-analogs **19–21** displayed improved permeability, while, surprisingly, also other ADME parameters such as solubility and *in vivo* clearance could be improved leading to a significantly enhanced oral exposure. At the same time, target potency was retained, thus demonstrating that fluorine, when properly placed within a scaffold, can be a highly useful tool for improvement of physicochemical and ADME properties while not affecting target binding. In addition, we believe that these new analogs can be valuable for further exploration of MK2 biology *in vivo*.

Table 2. Fluoro-Containing Pentacyclic MK2 Inhibitors Showed Improved Physicochemical and Pharmacokinetic Parameters Whilst Preserving Target Potency

Compd.	Structure	MK2 <sup>a</sup> [μM]	phsp27 <sup>b</sup> [μM]	TNFα <sup>c</sup> [μM]	TNFα whole blood <sup>d</sup> [μM]	log PAMPA <sup>e</sup> [cm/s]	logD <sub>7.4</sub>	BAV <sup>f</sup> [%]	AUC po dn <sup>g</sup> [nmol.h/L]	CL <sup>h</sup> [mL/min/kg]
1		<0.003	0.20	0.17	0.67	-5.2	3.4	27 ± 8	121 ± 35	100 ± 29
19		<0.003	0.37	0.55	1.65	-4.1	3.2	81 ± 34	3486 ± 1460	11 ± 5
20		<0.003	0.37	0.24	0.80	-4.5	3.3	60 ± 30	1301 ± 644	20 ± 8
21		<0.003	0.48	0.49	0.59	-4.8	2.8	77 ± 28	1766 ± 645	19 ± 6

IC<sub>50</sub> values determined as a mean ( $n \geq 2$ ) of <sup>a</sup>human MK2 inhibition, <sup>b</sup>inhibition of hsp27 phosphorylation in anisomycin stimulated THP-1 cells, <sup>c</sup>TNFα release inhibition in LPS stimulated human PBMCs, and <sup>d</sup>inhibition of LPS stimulated TNFα release from human whole blood. <sup>e</sup>Permeability determined by high-throughput PAMPA. <sup>f</sup>Oral bioavailability ± SD calculated as dose normalized ratio of extravascular AUC<sub>extrap</sub> to iv AUC<sub>extrap</sub> both parameter determined as a mean ± SD of 4 animals (female Sprague–Dawley rat). <sup>g</sup>Oral exposure measured as a mean ± SD of 4 animals (female Sprague–Dawley rat) after po dosing (3 mg/kg) using CMC/water/Tween (0.5:99:0.5) formulation, dn = dose-normalized to 1 mg/kg. <sup>h</sup>Clearance measured as a mean ± SD of 4 animals (Sprague–Dawley rat) after iv dosing (1 mg/kg) using NMP/PEG200 (30:70) formulation.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsmchemlett.8b00098](https://doi.org/10.1021/acsmchemlett.8b00098).

Description of *in vitro* assays, pharmacokinetic measurements, synthesis procedures and characterization data for all compounds, and UPLC and NMR charts (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

BAV, bioavailability; CMC, carboxymethylcellulose; DIPEA, ethyldiisopropyl amine.

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