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Implications for selectivity of 3,4-diarylquinolinones as p38αMAP kinase inhibitors

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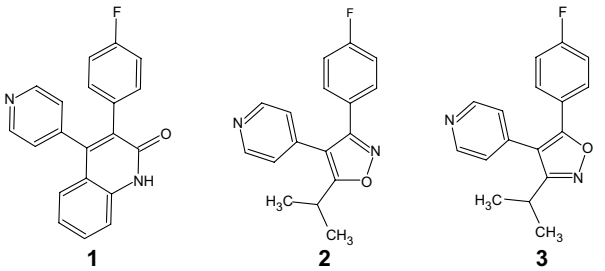
Abstract—In this study we report on the specificity profiling of the MAP kinase inhibitors **1**, **2**, and **3** in a panel of 78 protein kinases including the MAPK isoforms p38(α,β,γ,δ), JNK1/2/3, and ERK1/2/8 showing 3-(4-fluorophenyl)-4-pyridin-4-ylquinolin-2(1*H*)-one (**1**) to be highly selective for p38αMAPK with an IC_{50} of 1.8 μM. In contrast, besides p38α the isoxazoles **2** and **3** significantly inhibited JNK2/3 and further kinases beyond the MAPK family such as PKA, PKD, Lck, and CK1. By using sequence alignment and homology models of different members of the MAPK family the binding mode determining selectivity of **1** for the p38α isoform was investigated. For lead optimization of **1** a straightforward tandem-Buchwald-aldol synthetic approach toward the flexible decoration of the quinolin-2(1*H*)-one scaffold was employed. SAR for derivatives of **1** at the isolated p38αMAPK are presented.

The well-characterized mitogen activated protein kinases (MAPK) are a group of protein kinases (PK) that play an essential role in signal transduction of diseases such as in inflammation.[1] In humans, at least 11 members of the MAPK superfamily are known which can be divided into 6 groups: extracellular signal-regulated protein kinases (ERK1, ERK2); c-Jun N-terminal kinases (JNK1, JNK2, JNK3); p38s (p38-alpha, p38-beta, p38-gamma, p38-delta); ERK5; ERK3s (ERK3, p97-MAPK, ERK4); and ERK7s (ERK7, ERK8). Among them especially p38αMAPK (p38α) and JNK3 are considered to be promising targets for drug development.[2] In general the potency as well as the selectivity of PK inhibitors are important criteria.[3] However, in our former study compound **1** as an inhibitor of p38α (IC_{50} = 1.8 μM) was found to be selective over the closely related JNK3 whereas the two isoxazol-isomers **2** (p38α IC_{50} = 0.45 μM; JNK3 IC_{50} = 0.54 μM) and**3** (p38α IC_{50} = 2.2 μM; JNK3 IC_{50} = 3.5 μM) inhibited both enzymes.[4]

In this communication we report on the profiling of **1**, **2**, and **3** (Fig. 1) in a panel of 78 PKs[5,6] including the p38MAPK isoforms (α,β,γ,δ), JNK1/2/3 and ERK1/2/8 (Table 1; the complete profile over 78 PK and assay details are available on SI). Herein, compound **1** significantly inhibited only the p38α isoform whereas **2** and **3** additionally blocked JNK2/3 (**2** IC_{50} JNK2 = 1.28 μM). Furthermore besides MAPK, **2** markedly inhibited PKA[7] (28% ± 2), PKD1[8] (60% ± 1), Lck[9] (42% ± 0), and CK1δ[10,11] (6% ± 2).

Compound **3** secondary blocked Aurora B (22% ± 2), Aurora C[12] (35% ± 3), and CK1δ[10] (55% ± 8). However, since **1** (but not **2** and **3**) was selective for p38α in this panel we were particularly interested in differences of the binding modes in members of the MAPK family.

To address this question, rational binding modes of **1**, **2**, and **3** were modeled in the ATP binding pocket of p38α



1 **2** **3**

Figure 1. Structures of MAPK inhibitors **1**, **2**, and **3** possessing a vicinal 4-fluorphenyl/pyridine pharmacophore.

Keywords: p38MAP kinase; Inhibitors; Selectivity.
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Table 1. Activity profile of **1**, **2**, and **3** at a concentration of 10 μM over 10 PK of the MAPK superfamily represented as % residual activity of PK (and SD, $n = 3$) compared to control

	1	SD	2	SD	3	SD
ERK1	84	2	92	8	87	7
ERK2	93	3	86	1	88	1
JNK1	86	6	67	1	98	0
JNK2	89	6	13	1	59	6
JNK3	106	4	28	0	63	2
p38α	53	4	20	6	50	4
p38β	97	7	95	13	98	7
p38γ	89	3	84	0	94	9
p38δ	91	9	86	6	95	3
ERK8	73	10	84	6	88	6

(Fig. 2). According to the typical binding mode of compounds bearing a vicinal 4-F-phenyl/pyridine pharmacophore in p38α an H-bond from Met109 (hinge region) is addressed to the pyridine nitrogen. The 4-F-phenyl moiety is situated in the hydrophobic pocket II with the vicinal 4-F-phenyl/pyridine system clasping around 'gatekeeper'[13] residue Thr106 and thereby accounting for p38(α)-selectivity.

Furthermore an H-bond from Lys53 toward the carbonyl-oxygen (**1**) and to the isoxazole ring-nitrogen (**2**) or -oxygen (**3**) is formed, respectively. In light of the compound's differentiated inhibitory profile we compared these key residues by a sequence alignment of the highly conserved ATP binding pockets of MAPK (shown in SI) and their homology models (Fig. 3). The relevant residues Met and Lys are overall conserved while the gatekeeper differs within these enzymes. The role of more spacious Met146 in JNK3 compared to Thr106 in p38α was found to determine selectivity of six-membered core compound **1** for p38α but not for the five-membered core compounds **2** and **3**.[4] Actually p38α and p38β show a Thr residue as gatekeeper whereas in line with the hypothesis a Met (p38γ/δ and JNK1/2/3) or even bulkier residues (Gln ERK1/2, Phe ERK8) are situated at this position (Fig. 3). Thus, with the exception of p38β the loss of activity of **1** for these MAPK can be sufficiently explained by the major gatekeeper preventing access of **1** to these binding pockets. Although sharing the same gatekeeper residue 106 the only difference in the ATP pocket between the highly

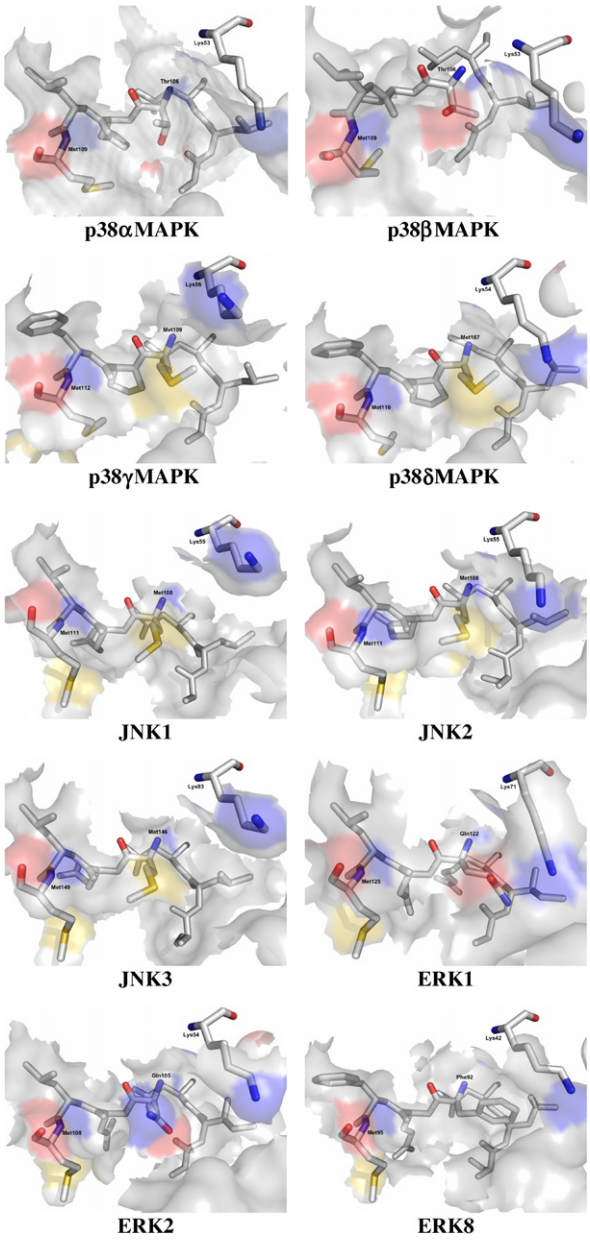


Figure 3. ATP binding pockets of p38α (1A9U),[14] JNK3 (1PMN)[15], and homology models for p38β/γ/δ, JNK1/2, and ERK1/2/8. Key residues are labeled (experimental details are given in supporting information).

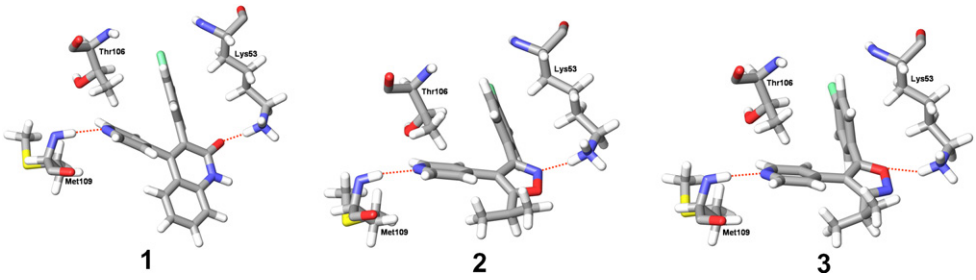


Figure 2. Modeled binding modes of **1**, **2**, and **3** in the ATP binding pocket of p38αMAPK (PDB 1AU9).[14] Key residues and ligand–protein interactions are shown.

homologous p38α/β isoforms is to be seen at position 107 (p38α His, p38β Thr).

To investigate if the different residues at position 107 could have an impact on the conformation of the neighboring gatekeeper Thr106 we compared the conformations of these amino acids in p38α crystal structures (Fig. 4).[16,17]

Herein, Thr106 is involved in water mediated H-bond interactions thereby significantly affecting the orientation of this gatekeeper residue. Typically, the methylene moiety of Thr106 is situated inside the pocket interacting with the ligand whereas the Thr106-OH is fixed in the backbone background to His107 (which is unique to p38α) via two water molecules (Fig. 4, left). In contrast, p38α without a ligand is showing a flip of Thr106 (and Met108) with the Thr-OH subsequently situated in the pocket and not involved in water mediated interactions (Fig. 4, right). Hence a considerable impact of His107 on the conformation of the gatekeeper Thr106 by ligand binding in p38α can be revealed. Concerning Thr107 in p38β instead of His107/p38α may have a significant consequence on the conformation of gatekeeper Thr106 in p38β and subsequently on ligand binding, too. However, this hypothesis is supported by the complete loss of activity of **1**, **2**, and **3** for p38β (and p38γ/δ, Table 1).

In terms of selectivity for p38α the situation for **2** and **3** seems to be more complex and further PK were inhibited (see above). As previously described[4] the highly homologous binding pockets of p38α, JNK3 (and JNK2) could account for the inhibition of these kinases by **2** and **3**. Interestingly, the compounds did not inhibit p38β while comparable p38α inhibitors such as SB203580 and SB202190 bearing the same prototypical vicinal pyridine/4-F-phenyl pharmacophore also have been shown to block p38β with similar potency.[5,6] However, determining a X-ray structure of a complex of **2** in p38α showing the decided protein conformation and ligand–protein interactions may answer this discrepancy.

Furthermore, the strength of the H-bond from the lysine residue of p38α and JNK2/3 addressing the isoxazole ring nitrogen in **2** was reported to be higher than the H-bond to the corresponding oxygen in **3**.[18] This is reflected by the relatively better inhibition of JNK2/3 and p38α of **2** compared to **3** (for IC_{50} values, see Table 1).

For the efficient optimization of the lead structure **1** showing promising selectivity for p38α we used a general synthetic approach toward the quinolin-2(1*H*)-one system by combining a Buchwald amidation reaction and an aldol condensation (Fig. 5).[19,20]

The quinolin-2(1*H*)-one-NH was not interacting to an amino acid residue in the calculated binding mode of **1** in the ATP binding pocket of p38α (Fig. 2). Thus, in order to reveal SAR data for this side a variety of substituents were introduced to the quinolin-2(1*H*)-one-nitrogen by S_N reaction of deprotonated **1** and alkylhalogenide.[21] Due to the tautomeric situation in the quinolinone nucleus[22] during this reaction both N-and O-alkylated products were obtained (Table 2).[23]

Furthermore, the 4-F-phenyl system of **1** was replaced by an *ortho*-toluyl moiety (**18**) to investigate the restriction of the conformation of this aromatic residue on the biological activity. In the S_N reaction of **18** to generate **19** the corresponding O-alkylated compound **20** (2-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]-3-(2-methylphenyl)-4-pyridin-4-ylquinoline) was generated. Compound **23** (4-pyridin-4-ylquinolin-2(1*H*)-one) was synthesized via an intramolecular $S_E$reaction of **22** (see SI) using PPA[24] and compound **25** (3,4-diphenyl-quinolin-2(1*H*)-one) obtained via aldol condensation of **24** (see SI).[4] The structures of compounds **9** (CCDC Nr 670663), **13** (CCDC Nr 670664), **14** (CCDC Nr 670665), and **19** (CCDC Nr 670666) have been proven by X-ray analysis (further synthetic and analytical details for all compounds are available on SI).

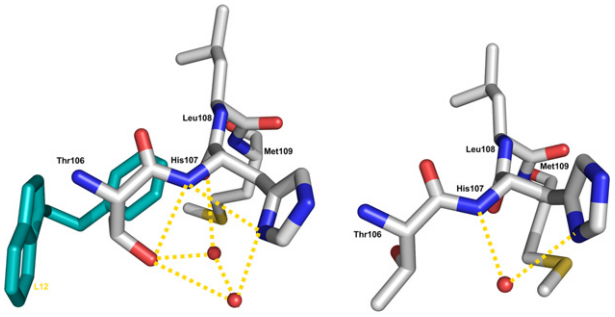


Figure 4. Exemplified different H-bond networks in p38α crystal structures involving water molecules (red dots) affecting the conformation of gatekeeper Thr106 and residue His107 in the ATP binding pocket (left, PDB 1W84[16] with ligand L12 in cyan; right, PDB 1P38,[17] no ligand bound).

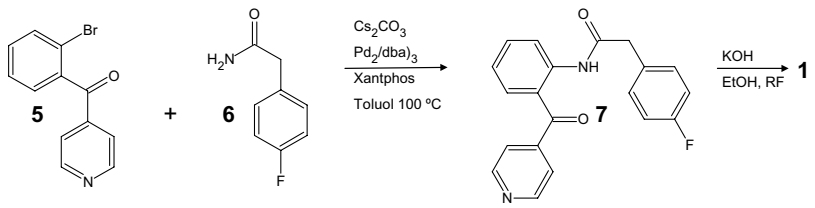
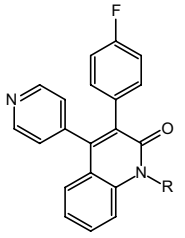
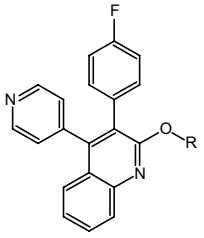
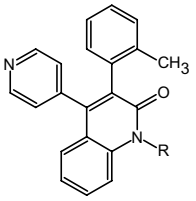
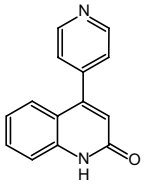
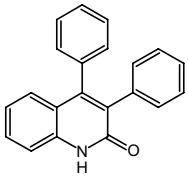


Figure 5. Preparation of **1** by a tandem Buchwald-aldol-reaction approach: compounds **5** and **6** are reacted by a Buchwald amidation to generate intermediate **7** which subsequently yields the quinolinone ring by an aldol condensation.

Table 2. Substitution patterns and indication of compounds

R			
–H	**1**	—	**18**
–Methyl	**8**	—	—
–Ehyl	**9**	**10**	—
–Propinyl	**11**	**12**	—
–Phenylethyl	**13**	**14**	—
–[(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl]	**15**	**16**	**19**
Butyl-1,2-diol	**17**	—	**21**
			
	23	**25**	

The compounds have been evaluated in vitro for their potency against p38α[25] (Table 3).

Within this series **1** was found again to inhibit the p38α enzyme most potent with an IC_{50} value of 1.8 μM.[4] The quinolinone-N-alkylated compounds **8**, **9**, **11**, and **13** showed slightly less inhibition activity yet still in the single digit μM range. In contrast to a promising modeled binding mode of **17** in the ATP pocket of p38α (not shown), the introduction of the two hydroxyl moieties in the quinolinone-*N*-alkyl side chain (**17**) slightly decreased the activity and hence this optimization strategy for **1** was not successful. However, accurate and actual virtual design of protein kinase inhibitors remains challenging.[26] As expected from our modeling data, the quinolinone O-alkylated compounds **10**, **12**, **14**, and **16** were inactive presumably mainly due to clashes with Lys53 in the ATP binding pocket (Fig. 2). Compound **18** was determined to have an IC_{50} value of approximately 10 μM and the introduction of the functionalized side chain (**21**) did not significantly change the activity, as already seen for the related derivatives **1**/**17**. Furthermore, the vanished activity of **23** on the one hand and **25** on the other hand indicated the vicinal pyridine/4-F-phenyl system in **1** to be favorable for p38α inhibition.

Taken together, **1** is a promising lead compound for the development of potent and selective p38α inhibitors.

Table 3. Inhibition of p38α activity by test compounds reported as IC_{50} values (μM) or % residual activity of the enzyme at a concentration of 10 μM

#	IC_{50} p38α (μM)	#	IC_{50} p38α (μM)
1	1.8	**18**	9.7
8	5.2	**19**	nd
9	2.2	**20**	nd
10	66% at 10 μM	**21**	80% at 10 μM
11	3.1	**23**	78% at 10 μM
12	79% at 10 μM	**25**	64% at 10 μM
13	8.2		
14	—		
15	55% at 10 μM		
16	—		
17	57% at 10 μM		

— Indicates no significant inhibition at 10 μM compound concentration. nd, not determined.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.12.073.

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20. General method for the preparation of quinolin-2(1*H*)-ones, exemplified by the synthesis of **1**. The reaction was carried out under argon. Compound **5** (900 mg, 3.43 mmol), (4-fluorophenyl) acetamide (630 mg, 4.11 mmol), Cs_2CO_3 (1.57 mg, 4.82 mmol), $Pd_2(dba)_3$ (32 mg, 0.03 mmol), and Xantphos (58 mg, 0.103 mmol) were dissolved in 5 ml of dry toluol and refluxed for 18 h. After cooling 20 ml of water was added and extracted by 2× 50 ml CH_2Cl_2. The organic phase was evaporated and the crude mixture of products **7** and **1** was redissolved in ethanol. After addition of 20 mg KOH the mixture was refluxed for 30 min, then water added and extracted with 2× 50 ml CH_2Cl_2. The organic phases were dried over Na_2SO_4, evaporated, and the product purified by column chromatography to yield 451 mg (42%) of **1** as a white solid.
21. General method for the S_N of compound **1**. In a dry 50 ml three-necked round-bottomed flask 1 mmol of **1** and 2 mmol of a 60% NaH suspension were stirred for 30 min at rt in 5 ml dry DMSO. Through a septum 2 mmol of the corresponding halogenalkyl was added via syringe and the mixture stirred for 60 min. Water (20 ml) was added, the reaction mixture extracted by 3× 30 ml ethyl acetate, the organic phase was separated, dried over Na_2SO_4, and evaporated. The N- (and O-) alkylated product(s) were purified by flash chromatography.
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