Towards the improvement of the synthesis of novel 4(5)-aryl-5(4)-heteroaryl-2-thio-substituted imidazoles and their p38 MAP kinase inhibitory activity†

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A series of 2-alkylsulfanyl-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)-substituted imidazoles was prepared and interaction possibilities of the 2-thioether moiety with phosphate/ribose binding pockets of p38 MAP kinase were investigated. Introduction of the alkyl/benzyl amino function at the pyridine moiety was carried out *via* nucleophilic substitution or *via* palladium catalyzed aryl-C–N-bond formation.

Introduction

p38 mitogen-activated protein (MAP) kinase, a serine/threonine kinase, is required for the biosynthesis and release of the pro-inflammatory cytokines IL-1 and TNF-α.[1] p38 MAP kinases are activated by infection or cellular stress such as ultraviolet light, heat or osmotic shock.[2] Inhibition of the p38 MAP kinase could reduce the expression of these cytokines. Therefore this may be a promising target for the treatment of many inflammatory disorders such as rheumatoid arthritis and inflammatory bowel disease. Pyridinyl imidazoles like the prototype inhibitor SB 203580 **1** (Fig. 1) are well-known ATP competitive p38 MAP kinase inhibitors.[3,4]

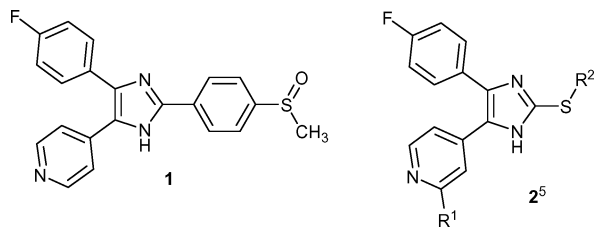


Fig. 1 Trisubstituted pyridinyl imidazoles.

We previously reported a series of trisubstituted imidazoles that are structurally related to **1**, notably 2-alkyl/benzylsulfanyl-4-fluorophenyl-5-pyridinylimidazoles **2** as potent p38 MAP kinase inhibitors (Fig. 1).[5] These 2-thioimidazoles are superior compared to the prototype SB-like 2-arylimidazoles because they have fewer interactions with metabolic enzymes like CYP-450.[5]

In a continuous effort to develop improved p38 MAP kinase inhibitors, we focused our attention on the optimization of substitution on the pyridinyl and imidazole rings. The 2-thioether moiety of **2** was investigated as a linker region for residues able to interact with the ribose pocket and/or phosphate binding region of the enzyme. Furthermore, the introduction of a secondary amino functionalization on the 2-position of the pyridinyl ring was investigated.

Herein, we wish to report the structure–activity relationship of a series of 2-alkylsulfanyl-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)-substituted imidazoles (**10a–u**) and a convenient, straightforward synthetic route to 4-aryl-5-heteroaryl-substituted imidazole-1,3-dihydro-2-thiones (**9a–h**).

The 2-thioether moieties at the imidazole core, exemplified by 2,3-dihydroxypropyl (Fig. 2), has improved the p38 MAP kinase inhibition, most likely by targeting the ribose pocket and/or phosphate binding region of the ATP binding site of p38.

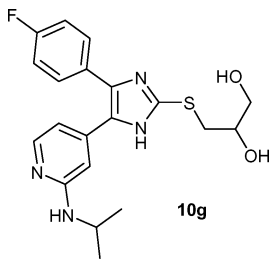
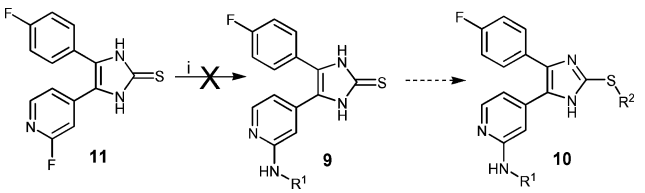


Fig. 2 Kinase inhibitor **10g**.

Results and discussion

Synthesis

The first attempted synthetic approach to the title compounds is outlined in Scheme 1. The starting point of the synthesis is 4-(4-fluorophenyl)-5-(2-fluoropyridin-4-yl)-1,3-dihydroimidazol-2-thione (**11**), which was obtained from 2-fluoro-4-methylpyridine according to a 4-step synthesis described in previous publications.[5,6] Initial steps to **10** were nucleophilic aromatic substitutions on the pyridinyl moiety with alkyl or benzyl amines followed by alkylation of the sulfur atom. The critical point was the isolation of the thiones **9** accompanied by a poor reproducibility of the approach.



Scheme 1 *Reagents and conditions*: (i) R^1–NH_2 (10 equiv.), 150 °C.

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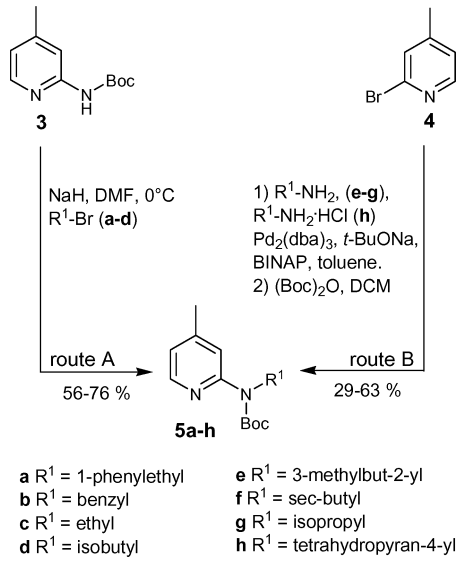
Starting from *N*-protected 2-(*N*-alkylamino)-4-methylpyridines we developed a new synthetic strategy, leading to the key intermediates 4-aryl-5-heteroaryl-substituted imidazole-1,3-dihydro-2-thiones **9a–h** (Schemes 2 and 3).



Scheme 2 Synthesis of the 2-(*N*-Boc-*N*-alkyl/phenylalkylamino)-4-methylpyridines **5a–h**.

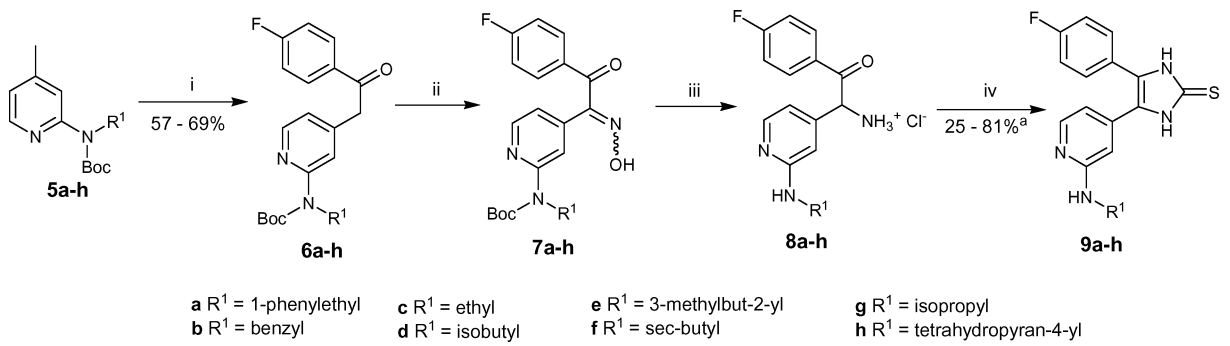
To protect the amino function, the Boc-protecting group was chosen because of its stability under strongly basic conditions and its facile cleavage under acidic conditions. The intermediate Boc-protected 2-(*N*-alkyl/phenylalkylamino)-4-methylpyridines **5a–h** were synthesized by two different routes (Scheme 2). In the case of benzylic and primary halides (**5a–d**) we started from 2-(*N*-Boc-amino)-4-methylpyridine **3** *via* nucleophilic substitution (route A). The carbamate **3** was prepared by reacting 2-amino-4-methylpyridine and di-*tert*-butyl dicarbonate in *tert*-butanol. The carbamate **3** was deprotonated using sodium hydride (1.25 equiv.) in dry DMF and treated with the alkyl/benzyl halide (1.15 equiv.) to afford **5a–d**.[7]

For secondary alkyl halides this method failed or gave poor yields. Therefore, route B was used for the synthesis of picolines **5e–h** (Scheme 2). The aryl-C–N-bond formation was achieved by a Buchwald–Hartwig reaction.[8,9] Thus, heating 2-bromo-4-methylpyridine (**4**) with alkyl amines (1.2 equiv.) in the presence of $Pd_2(dba)_3$ (2 mol%), BINAP (4 mol%) and *t*-BuONa (1.4 equiv.) in toluene yielded the corresponding 2-(alkylamino)-4-methylpyridines **5e–h**. These compounds could be Boc-protected without further purification. In some cases, especially when introducing small aliphatic substituents, a disubstituted 2-(*N*,*N*-dialkylamino)-4-methylpyridine was found as by-product. To react 4-aminotetrahydropyrano hydrochloride 2.4 equiv. of base was used.

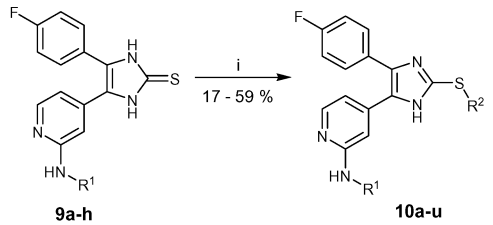
The straightforward synthesis of imidazole-2-thiones **9a–h** from picolines **5a–h** is presented in Scheme 3. Picolines **5a–h** were deprotonated with sodium hexamethyldisilazane (NaHMDS) in THF at 0 °C and reacted with ethyl 4-fluorobenzoate to afford ethanones **6a–h**. The reaction was quenched with aqueous NH_4Cl solution and purified by flash chromatography, thus allowing the recovery of unreacted starting material **5**. Upon treatment with sodium nitrite (3.0 equiv.) in acetic acid at 10 °C ethanones **6a–h** were converted into the α-hydroxyiminoketones **7a–h**. The oximes **7a–h** were obtained as colourless foams and as a mixture of isomers, indicated by proton NMR. In methanolic hydrogen chloride, the oxime functionality was reduced in the presence of Pd/C under a hydrogen atmosphere at room temperature and atmospheric pressure to an amino group. This step was accompanied by cleavage of the Boc-group as well as by transfer of the amines into the corresponding amine hydrochlorides **8a–h**. The hydrochlorides **8a–h** could be used in the next step without further purification. Cyclisation to imidazole thiones was achieved by heating **8a–h** and potassium thiocyanate in DMF to reflux temperature. After cooling to room temperature and treatment with water, the imidazole-1,3-dihydro-2-thiones **9a–h** precipitated as yellowish solids in good yield and purity. Finally, the sulfur atom was functionalized *via* nucleophilic substitution to compounds **10a–u** (Scheme 4). Alkylation of **9a–h** with various hydrophilic alkyl halides proceeded upon treatment with sodium ethoxide or potassium *tert*-butoxide in methanol.

Biological studies

The biological test results (p38[10] and TNF-α release) show that the prepared 4(5)-aryl-5(4)-heteroaryl-2-thio-substituted imidazoles **10a–u** are highly potent inhibitors with IC_{50} values in the low nanomolar range (Table 1). Compound **10g** inhibits the target enzyme *in vitro* 13 times more strongly than the prototype inhibitor SB 203580. These results indicate that substituents at both the



Scheme 3 Synthesis of the key intermediates 5-(2-(alkyl/phenylamino)pyridin-4-yl)-4-(4-fluorophenyl)-1,3-dihydroimidazol-2-thiones **9a–h**; *reagents and conditions*: (i) NaHMDS, ethyl 4-fluorobenzoate, THF, 0 °C–rt; (ii) $NaNO_2$, acetic acid, 10 °C–rt, 2.5 h; (iii) Pd/C 10%, MeOH–HCl, H_2, rt, 16 h; (iv) KSCN, DMF, reflux, 3 h; [a]yield over 3 steps.



a R^1 = 1-phenylethyl
b R^1 = benzyl
c R^1 = ethyl
d R^1 = isobutyl
e R^1 = 3-methylbut-2-yl
f R^1 = sec-butyl
g R^1 = isopropyl
h R^1 = tetrahydropyran-4-yl

a R^1 = 1-phenylethyl, R^2 = 2,3-dihydroxypropyl
b R^1 = benzyl, R^2 = 2,3-dihydroxypropyl
c R^1 = ethyl, R^2 = 2,3-dihydroxypropyl
d R^1 = isobutyl, R^2 = 2,3-dihydroxypropyl
e R^1 = 3-methylbut-2-yl, R^2 = 2,3-dihydroxypropyl
f R^1 = sec-butyl, R^2 = 2,3-dihydroxypropyl
g R^1 = isopropyl, R^2 = 2,3-dihydroxpropyl
h R^1 = tetrahydropyran-4-yl, R^2 = 2,3-dihydroxypropyl
i R^1 = 3-methylbut-2-yl, R^2 = 2-hydroxyethyl
j R^1 = 3-methylbut-2-yl, R^2 = 3-hydroxypropyl
k R^1 = 3-methylbut-2-yl, R^2 = (R)-2,3-dihydroxypropyl
l R^1 = 3-methylbut-2-yl, R^2 = (S)-2,3-dihydroxypropyl
m R^1 = 3-methylbut-2-yl, R^2 = methylacet-2-yl
n R^1 = isopropyl, R^2 = (R)-2,3-dihydroxypropyl
o R^1 = isopropyl, R^2 = (S)-2,3-dihydroxypropyl
p R^1 = isopropyl, R^2 = methylacet-2-yl
q R^1 = 1-phenylethyl, R^2 = methylacet-2-yl
r R^1 = 1-phenylethyl, R^2 = carboxymethyl
s R^1 = 1-phenylethyl, R^2 = (diethoxyphosphoryl)methyl
t R^1 = isopropyl, R^2 = 2-hydroxyethyl
u R^1 = tetrahydropyran-4-yl, R^2 = 2-hydroxyethyl

Scheme 4 Synthesis of the target compounds **10a–u**; *reagents*: (i) R^2–X (X = Cl, Br, I), *t*-BuOK or EtONa, MeOH.

Table 1 Evaluation of the prepared compounds **10a** and **10g** for p38 MAP kinase inhibitory activity and TNF-α release in human whole blood

Test compound	$IC_{50} \pm$ SEM/μM p38[a]	$IC_{50} \pm$ SEM/μM TNF-α[a]
10a	0.049 ± 0.009 (2)	0.936 ± 0.21 (2)
10g	0.003 ± 0.001 (3)	0.126 ± 0.0081 (3)
SB 203580	0.038 ± 0.011 (14)	1.79 ± 0.35 (14)

[a] Number of experiments is given in brackets.

2-thioether moiety and the 2-amino position of the pyridine have effects on the potency of the inhibitory activity of the compounds.

In particular, substitution at the 2-position of the imidazole core may allow hydrogen bonding with the ribose and phosphate binding pockets of p38, thus enhancing inhibitory properties of the compounds.

In Fig. 3, the suggested binding mode for **10g** is reported. Noteworthy is the interaction possibility of the 2,3-dihydroxypropyl residue with the p38 conserved residues Asp168-Phe169-Gly170 (DFG), in particular the hydrogen bonding with Asp168.

Conclusions

In summary, we report a straightforward access to the 2-(alkyl/benzylamino)pyridinyl substituted imidazole-1,3-dihydro-2-thiones **9a–h** and their functionalization to potent p38 MAP kinase inhibitors **10a–u**.

Residues with polar groups at the 2-thioether moiety, which may interact with the ribose and/or phosphate pocket of the enzyme, are well tolerated.

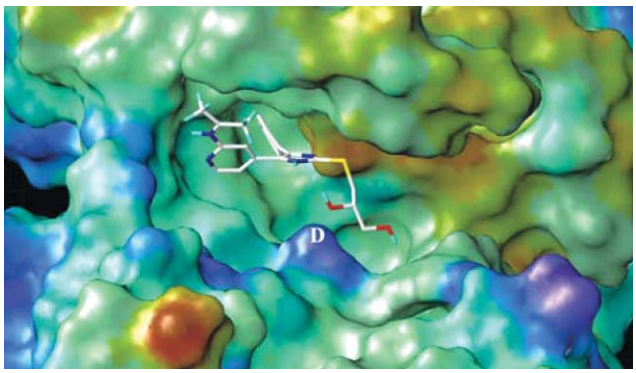


Fig. 3 Suggested binding mode for **10g**. Asp168 is labelled with a white D. After geometric optimization in the MMFF94 force field, the molecule has been docked in the p38 active centre by using the docking program FlexX. As the protein model, the X-ray structure 1bl7.pdb[11] had been used.

Acknowledgements

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