Organic & Biomolecular Chemistry

PAPER



Cite this: Org. Biomol. Chem., 2015, 13, 10699

An optimized and versatile synthesis to pyridinylimidazole-type $p38\alpha$ mitogen activated protein kinase inhibitors[†]

Ahmed El-Gokha,^{a,b} Stefan A. Laufer^c and Pierre Koch*^a

An optimized strategy for the synthesis of the potent $p38\alpha$ mitogen-activated protein kinase inhibitors 2-(2-hydroxyethylsulfanyl)-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)imidazole (**3**) and 2-(2,3-dihydroxypropylsulfanyl)-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)imidazole (**4**) starting from 2-fluoro-4-methylpyridine is reported. In contrast to a previously published synthesis starting from 2-bromo-4-methylpyridine, the overall yield could be increased from 3.6% to 29.4%. Moreover, this strategy avoids the use of palladium as a catalyst and is more diverse and versatile. Using this optimized protocol, both enantiomers of potent inhibitor **3** were synthesized. Biological data demonstrated that the (*S*)-enantiomer is the two times more potent eutomer.

Received 21st July 2015, Accepted 3rd September 2015 DOI: 10.1039/c5ob01505g

www.rsc.org/obc

Introduction

The p38α mitogen-activated protein (MAP) kinase is a serine/ threonine kinase, which links extracellular signals to the intracellular machinery modulating a plethora of cellular processes, e.g., the release of pro-inflammatory cytokines like tumor necrosis factor- α (TNF- α) and interleukin-1 β .¹ Therefore, the inhibition of p38a MAP kinase was evaluated as a therapeutic strategy for the treatment of cytokine-driven diseases like rheumatoid arthritis or psoriasis. Other studies suggest an important role of p38a MAP kinase in the pathogenesis of neurodegenerative diseases like Parkinson's disease, Alzheimer's disease or multiple sclerosis.²⁻⁴ Recently, several clinical trials investigating inhibitors of p38a MAP kinase for the treatment of chronic obstructive pulmonary disease were terminated.5,6 Trisubstituted pyridinylimidazoles, like prototypical inhibitor SB203580 (1) or ML3403 (2), are amongst the most prominent adenosine triphosphate (ATP) competitive inhibitors of p38 α MAP kinase (Fig. 1).⁷⁻⁹

In 2008, we reported 2-(2-hydroxyethylsulfanyl)-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)imidazole (3) and 2-(2,3-



View Article Online

Fig. 1 Pyridinylimidazole based $p38\alpha$ MAP kinase inhibitors.

dihydroxypropylsulfanyl)-4-(4-fluorophenyl)-5-(2-aminopyridin-4-yl)imidazole (4) as optimized p38 α MAP kinase inhibitors derived from ML3403 (Fig. 1).¹⁰ The hydroxyl containing moie-

^aInstitute of Pharmaceutical Sciences, Department of Medicinal and Pharmaceutical Chemistry, Eberhard Karls Universität Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Germany. E-mail: pierre.koch@uni-tuebingen.de

^bMenofia University, Chemistry Department, Faculty of Science, Menofia, Egypt ^cInstitute of Pharmaceutical Sciences, Chair for Medicinal and Pharmaceutical Chemistry, Eberhard Karls Universität Tübingen, Auf der Morgenstelle 8, 72076 Tübingen, Germany

[†]Electronic supplementary information (ESI) available: Schemes S1–S7, experimental procedures and analytical data, copies of ¹H NMR and ¹³C NMR. See DOI: 10.1039/c5ob01505g

Paper

ties at the imidazole C²-position may interact with the ribose and the phosphate binding site of the enzyme. In contrast to the parent compound ML3403, compounds 3 and 4 show improved p38 α MAP kinase inhibitory activity and a strong increase in inhibition of LPS-stimulated TNF- α release from human whole blood (in case of 3, >110-fold increase compared to 2). In order to profile these inhibitors in further *in vitro* and *in vivo* experiments, a high yielding and scalable synthesis is required.

The previously reported synthetic route toward inhibitors 3 and 4 starts from 2-bromo-4-methylpyridine (5) and is depicted in Scheme 1.^{10,11} In the first step of this synthesis, the amino moiety was introduced at the pyridine C²-position via Buchwald-Hartwig arylamination and the obtained secondary amine was directly protected with the Boc-group. Picoline 6 was converted into the ethanone 7 by reaction with ethyl 4-fluorobenzoate using NaHMDS as a base. Upon treatment with an excess of sodium nitrite in acetic acid, ethanone 7 was converted into the α -hydroxyiminoketone 8. Reduction of oxime 8 to a corresponding amine hydrochloride 9 accompanied by deprotection of the Boc-group was achieved by hydrogenation in methanolic hydrogen chloride using Pd/C as a catalyst. Cyclization was accomplished by treatment with potassium thiocyanate. Finally, the thione 10 was converted into the target compounds 3 and 4 by nucleophilic substitution reactions with 2-bromoethanol and 3-bromopropane-1,2-diol, respectively. The total yield of this synthetic strategy to pyridinylimidazoles 3 and 4 starting from 2-bromo-4-methylpyridine (5) is 3.6% and 3.9% (7 linear steps), respectively.

The major limitations of this synthetic strategy for scale-up are the introduction of the amino moiety in the first step of the synthesis, which requires the addition and removal of a protecting group and its low overall yield (>4%). Moreover, the required early stage use of the palladium catalyst is quite cost intensive due to the higher amount needed in scale-up.

Another attempted synthetic strategy to the target compounds 3 and 4 reported from our group is depicted in Scheme 2.12 The introduction of the 3-methyl-2-butylamino moiety at the pyridine C²-position as well as the moiety at the imidazole C^2 -S-position was envisaged in the last two steps of the synthesis. The key compound for this route 4-(4-fluorophenyl)-5-(2-fluoropyridin-4-yl)-1,3-dihydroimidazole-2-thione (12) was prepared according to a protocol from Laufer and coworkers^{8,13} in four steps starting from 2-fluoro-4-methylpyridine (11) in an overall yield of 57% (for details, see Scheme S1 in the ESI[†]). The alkylsulfanyl moiety was introduced by nucleophilic substitution of thione 12 and the appropriate alkyl halide. In the last step, the fluorine atom in 13a and 13b should be displaced by 3-methyl-2-butylamine (14) to obtain 3 and 4, respectively. By heating 13a or 13b with the amine 14, we observed an unexpected transformation of 2-alkylsulfanylimidazoles bearing a 2-hydroxyethyl or a 2,3dihydroxypropyl moiety at the imidazole C²-S-position into the imidazol-2-one derivative 15.

Herein, we report a distinct and optimized procedure to prepare pyridinylimidazoles **3** and **4** in higher yields and with increased versatility compared to the previously published



Scheme 1 Reported synthesis of pyridinylimidazoles 3 and 4. Reagents and conditions: (i) 1. 3-methyl-2-butylamine (rac.), $Pd_2(dba)_3$, t-BuONa, BINAP, toluene, reflux temperature; 2. Boc_2O , DMAP, CH_2Cl_2 , r.t.; (ii) NaHMDS, ethyl 4-fluorobenzoate, THF, 0 °C – r.t.; (iii) NaNO₂, acetic acid, 10 °C – r.t.; (iv) Pd/C 10%, MeOH/HCl, H_2 , atmospheric pressure, r.t.; (v) KSCN, DMF, reflux temperature; 3 h; (vi) 2-bromoethanol, t-BuOK, reflux temperature; 1.5 h in case of compound 3 or 3-bromopropane-1,2-diol, t-BuOK, reflux temperature; 3 h in case of compound 4; ^ayield over three steps, compounds 8 and 9 were not isolated.



Scheme 2 Unexpected reaction of 2-alkylimidazoles 13a and 13b into imidazol-2-ones. Reagents and conditions: (i) EtoNa, methanol, 2-bromoethanol, r.t., 4 h (for synthesis of 13a) or EtoNa, methanol, 3-bromopropane-1,2-diol, r.t., 16 h (for synthesis of 13b); (ii) 160 °C, 10 h, sealed tube.

protocols. Moreover, the described synthetic strategy avoids the use of palladium as a catalyst for introduction of the amino moiety at the pyridine C^2 -position.

Results & discussions

In order to avoid the aforementioned conversion of 2-alkylsulfanylimidazoles bearing a 2-hydroxyethyl or a 2,3-dihydroxypropyl moiety at the imidazole C^2 –S-position into the corresponding imidazol-2-one derivatives, we pursued the strategic introduction of a hydroxyl protecting group at the imidazole C^2 -S moiety prior to this step and its cleavage in the subsequent step (Scheme 3).

Initially, we attempted to make use of an acetyl ester to protect the hydroxy function (Scheme 4).¹⁴ Therefore, imidazole-2-thione **12** was treated with 2-bromoethyl acetate under mildly basic conditions in order to obtain ester **16**, bearing the acetyl protected hydroxyethyl moiety at the imidazole C^2 -Sposition. Compound **16** was reacted with 3-methyl-2-butylamine in a nucleophilic aromatic substitution reaction. Under these harsh and basic conditions (160 °C, excess of amine), the acetyl protecting group was cleaved and imidazol-2-one **15** was isolated as the major product of this reaction. Decrease of



Scheme 3 Retrosynthetic approach for synthesis of 3 (PG, protecting group).



Scheme 4 Conversion of ester 16 to imidazol-2-one 15. Reagents and conditions: (i) 2-bromoethyl acetate, Cs₂CO₃, DMF, 55 °C, 30 min; (ii) 3-methyl-2-butylamine (rac.), 160 °C, 16 h.



Scheme 5 Optimized synthesis of 3 using THP as the hydroxyl protecting group. Reagents and conditions: (i) t-BuONa, methanol, 2-(2-bromoethoxy)-tetrahydro-2*H*-pyran, 30 min, 50 °C; (ii) 3-methyl-2-butylamine (rac.), 160 °C, 16 h, sealed tube; (iii) 1.25 M HCl/EtOH, 3 h, r.t.

both, reaction temperature and excess of amine resulted in a slow or no conversion.

As another protecting group for the hydroxy function, we used the acid labile but base stable tetrahydropyranyl (THP) group (Scheme 5).¹⁵ Imidazole-2-thione **12** was reacted with 2-(2-bromoethoxy)-tetrahydro-2*H*-pyran to give compound **17** in good to moderate yields. In the next step, the amino function at the pyridine C^2 -position was introduced *via* nucleophilic aromatic substitution under the above mentioned conditions. To our delight, we found the THP-group to be sufficiently stable and compound **18**, the THP-protected derivative of **3**, was obtained in good yields. Finally, treatment of **18** with ethanolic hydrochloric acid solution resulted in cleavage of the THP group and the target compound **3** was obtained in quantitative yields.

With a total yield of 29.4% in 7 linear steps, the optimized synthesis of compound 3 starting from 2-fluoro-4-methylpyridine (11) compares very well to the reported synthesis starting from 2-bromo-4-methylpyridine (5), giving more than eight times higher yields (compare Scheme 5 *vs.* Scheme 1). Using this protocol, 2.3 g of inhibitor 3 were synthesized starting from 3.0 g imidazole-2-thione 12 in similar yields as reported in Scheme 5 (for details, see ESI[†]).

For optimizing the synthesis of inhibitor **4**, we used an acetal group to protect the **1**,2-dihydroxy function.¹⁶ Acetals are generally stable under basic conditions and liberate the diol under acidic conditions. The optimized route to **4** is depicted in Scheme 6. The dihydroxypropylsulfanylimidazole **13b** was converted into the corresponding acetonide **19** by



Scheme 6 Optimized synthesis of 4 using acetal as the 1,2-dihydroxyl protecting group. Reagents and conditions: (i) *t*-BuONa, methanol, 3-bromopropane-1,2-diol, 70 °C, 1 h; (ii) acetone, *p*-TsOH, reflux temperature, 24 h; (iii) 3-methyl-2-butylamine (rac.), 160 °C, 16 h, sealed tube; (iv) 2 M aq. HCl, r.t., 3 h; ^ayield over two steps, compound **13b** was not isolated.

Paper



Scheme 7 Synthesis of pyridinylimidazoles S2, S4, S6, S8, S10 and S12. For reagents and conditions, see the ESI.†

treatment with acetone under *p*-toluenesulfonic acid (*p*-TsOH) catalysis. In the next step, the amino function was introduced at the pyridine C²-position *via* nucleophilic aromatic substitution and finally the acetal protecting group was cleaved with diluted hydrochloric acid to yield inhibitor **4**. The overall yield of this protocol starting from **11** is 20.2% (8 linear steps), which is five times higher than the previously reported synthesis starting from **5** (Scheme **1**, total yield: 3.9%).

Both optimized synthetic strategies presented in Schemes 5 and 6 are versatile and diverse since the introduction of both, the imidazole C^2 -S moiety and the pyridine C^2 -amino function are in the last steps of the sequence. Therefore, these strategies allow a facile and high-yielding access to further pyridinylimidazole derivatives (Scheme 7, Schemes S2-S7, ESI[†]). Starting from intermediates 17 or 19, both enantiomers of 3 (compounds S2 and S4), the hydroxyethylsulfanyl and dihydroxypropylsulfanyl derivatives of ML3403 (S6 and S8) and both enantiomers of S6 (compounds S10 and S12) were prepared. The synthesized pyridinylimidazoles were tested in an ELISA-assay for their ability to inhibit the p38a MAP kinase (Table 1).¹⁷ Furthermore, the potential to inhibit the LPS-stimulated TNF- α release from human whole blood was determined for S2 and S4, the most promising inhibitors in this set, and compared to the racemic mixture, compound 3. In case of the chiral inhibitors S2, S4, S10 and S12, the biological data revealed

Table 1 Biological data for selected pyridinylimidazoles

Cmpd	$IC_{50} \pm SEM$	
	p38α MAP kinase ^a	TNF- α release ^b
3 (LN950)	$11 \pm 1 \text{ nM}^c$	$37 \pm 4 \text{ nM}^c$
4 (LN941)	$15 \pm 2 \text{ nM}^c$	$183 \pm 16 \text{ nM}^{c}$
S2 [(R)-3]	$19 \pm 2 \text{ nM}$	$84 \pm 3 \text{ nM}$
S4 [(S)-3]	$14 \pm 2 \text{ nM}$	$20 \pm 3 \text{ nM}$
S6	$32 \pm 3 \text{ nM}$	nd^d
S8	$41 \pm 7 \text{ nM}^c$	$936 \pm 15 \text{ nM}^{c}$
S10 [(<i>R</i>)-6]	$42 \pm 1 \text{ nM}$	nd
S12 [(S)-6]	22 ± 0.2 nM	nd

^{*a*} Results are from three experiments. ^{*b*} Tests were carried out in duplicate. ^{*c*} Test data are from Koch *et al.*^{10 d} nd. not determined.

the (*S*)-enantiomer as the eutomer. Compared to the racemic inhibitor **3**, the LPS-stimulated TNF- α -release is inhibited two times more by the (*S*)-enantiomer **S4**, whereas, the (*R*)-enantiomer **S2** is as expected two times less active in this assay.

Conclusion

An optimized and diverse synthetic approach for the preparation of pyridinylimidazole-based p38 α MAP kinase inhibitors

is described. Compared to a previously reported method, an up to eight times higher overall yield was obtained.

A series of eight pyridinylimidazoles was synthesized using these optimized conditions. In case of the chiral inhibitor 3, biological evaluation showed that the (*S*)-enantiomer is the two times more potent eutomer.

Acknowledgements

S. Bauer, M. Goettert, D. Müller, and K. Bauer are gratefully acknowledged for their assistance in biology testing.

Notes and references

- Z. Chen, T. B. Gibson, F. Robinson, L. Silvestro, G. Pearson,
 B. E. Xu, A. Wright, C. Vanderbilt and M. H. Cobb, *Chem. Rev.*, 2001, 101, 2449–2476.
- 2 L. Munoz and A. J. Ammit, *Neuropharmacology*, 2010, 58, 561–568.
- 3 K. Namiki, H. Matsunaga, K. Yoshioka, K. Tanaka, K. Murata, J. Ishida, A. Sakairi, J. Kim, N. Tokuhara, N. Shibakawa, M. Shimizu, Y. Wada, Y. Tokunaga, M. Shigetomi, M. Hagihara, S. Kimura, T. Sudo, A. Fukamizu and Y. Kasuya, *J. Biol. Chem.*, 2012, 287, 24228–24238.
- 4 G. Wang, J. Pan and S. D. Chen, *Prog. Neurobiol.*, 2012, **98**, 207–221.

- 5 W. MacNee, R. J. Allan, I. Jones, M. C. De Salvo and L. F. Tan, *Thorax*, 2013, **68**, 738–745.
- 6 J. C. Betts, R. J. Mayer, R. Tal-Singer, L. Warnock, C. Clayton, S. Bates, B. E. Hoffman, C. Larminie and D. Singh, *Pharmacol. Res. Perspect.*, 2015, 3, e00094.
- 7 C. Peifer, G. Wagner and S. Laufer, *Curr. Top. Med. Chem.*, 2006, **6**, 113–149.
- 8 S. A. Laufer, G. K. Wagner, D. A. Kotschenreuther and W. Albrecht, *J. Med. Chem.*, 2003, **46**, 3230–3244.
- 9 S. Laufer, D. Hauser, T. Stegmiller, C. Bracht, K. Ruff,
 V. Schattel, W. Albrecht and P. Koch, *Bioorg. Med. Chem. Lett.*, 2010, 20, 6671–6675.
- 10 P. Koch, C. Bäuerlein, H. Jank and S. Laufer, *J. Med. Chem.*, 2008, **51**, 5630–5640.
- 11 S. Laufer and P. Koch, Org. Biomol. Chem., 2008, 6, 437-439.
- 12 P. Koch and S. Laufer, J. Med. Chem., 2010, 53, 4798-4802.
- 13 S. A. Laufer and A. J. Liedtke, *Tetrahedron Lett.*, 2006, 47, 7199–7203.
- 14 P. G. M. Green and T. W. Wuts, in *Protective Groups in Organic Synthesis*, John Wiley & Sons, Inc., New York, 3rd edn, 1999, pp. 150–160, 712–715.
- 15 P. G. M. Green and T. W. Wuts, in *Protective Groups in Organic Synthesis*, John Wiley & Sons, Inc., New York, 3rd edn, 1999, pp. 27–33, 708–711.
- 16 P. G. M. Green and T. W. Wuts, in *Protective Groups in Organic Synthesis*, John Wiley & Sons, Inc., New York, 3rd edn, 1999, pp. 207–215, 716–719.
- 17 M. Goettert, R. Graeser and S. A. Laufer, *Anal. Biochem.*, 2010, **406**, 233–234.