Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 3656–3660
Bioorganic & Medicinal Chemistry Letters

IRAK-4 inhibitors. Part III: A series of imidazo[1,2-*a*]pyridines

George M. Buckley, Richard Fosbeary, Joanne L. Fraser, Lewis Gowers, Alicia P. Higueruelo, Lynwen A. James, Kerry Jenkins,* Stephen R. Mack, Trevor Morgan, David M. Parry, William R. Pitt, Oliver Rausch, Marianna D. Richard and Verity Sabin

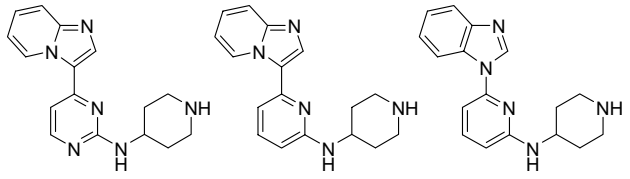
UCB, Granta Park, Great Abington, Cambridge CB21 6GS, UK

Received 12 March 2008; revised 15 April 2008; accepted 18 April 2008
Available online 24 April 2008

Dedicated to the memory of our friend and colleague David Rainey.

Abstract—Following the identification of a potent IRAK inhibitor through routine project cross screening, a novel class of IRAK-4 inhibitor was established. The SAR of imidazo[1,2-*a*]pyridino-pyridines and benzimidazolo-pyridines was explored.

In the preceding article we demonstrated how a cross screening hit from another kinase programme yielded the potent imidazo[1,2-*a*]pyridino-pyrimidine IRAK-4 inhibitor **1**.[1] This led to lead compounds **2** (imidazo[1,2-*a*]pyridino-pyridine) and **3** (benzimidazolo-pyridine) as our preferred scaffolds for optimisation.



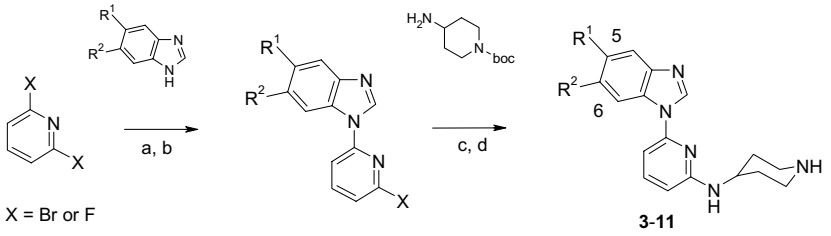
Initially the SAR of the 5,6-fused bicyclic binding group was studied on the benzimidazole analogue series due to the simplicity of the chemistry and readily available substituted starting materials.[2] A series of analogues were prepared from commercially available benzimidazoles and 2,6-difluoro or 2,6-dibromopyridine by sequential S_NAr displacement reactions and separation of regioisomers as required (Scheme 1).

Substitution in the 6-position (R^2) resulted in significant increase in IRAK-4 potency (5- to 25-fold) whereas substitution at the 5-position (R^1) reduced potency irrespective of whether the 6-position was substituted (Table 1). With this in mind we embarked on the more involved synthesis of imidazo[1,2-*a*]pyridino-pyridines substituted in the regio-equivalent position (Scheme 2 and Table 2). A variety of groups were tolerated in this position, many giving low nanomolar enzyme inhibition potencies (selected examples shown, **12**–**24**). The SAR was not very discernable except that the bulky tertiary amides were less potent than the primary amide (**23** < **24** < **22**). Homology model docking experiments of this series imply that the 6-substituent points towards the ATP-ribose region.[1] It is plausible that the flat SAR might be a result of the displacement of a bound water molecule from this site, thus gaining an entropic advantage in binding which would be largely independent of spatial complementarily or bonding interactions.

We also focused attention on the pyridine 2-position substituent. Much of the SAR could be probed by employing parallel synthesis (Scheme 3). The key bromopyridine intermediate **25** was prepared via Stille coupling[3] of the tri-*n*-butylstannane formed in situ from trans-metallation of the Grignard derived from 3-bromo imidazo[1,2-*a*]pyridine. A variety of N-linked derivatives were prepared using an automated serial microwave reactor,[4] either by direct S_NAr chemistry (method b) or by using Buchwald palladium catalysed

Keywords: IRAK; IRAK-4; IRAK-4 inhibitor; Kinase; Kinase inhibitor; Imidazo[1,2-*a*]pyridine; Inflammation; Anti-inflammatory; Ligand efficiency; IL-1; TNF-alpha; TNFα; Immunity.
* Corresponding author. Tel.: +44 1223 896400.; e-mail: drkerryjenkins@yahoo.co.uk

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.04.042



Scheme 1. Synthesis of substituted benzimidazolo-pyridines **3**–**11**. Reagents and conditions: (a) NaH, NMP, 180 °C, microwave (33–90%); (b) HPLC separation of regioisomers as required; (c) 4-amino-1-Boc piperidine, NMP, 140 °C, microwave (13–75%); (d) HCl, Et_2O, DCM.

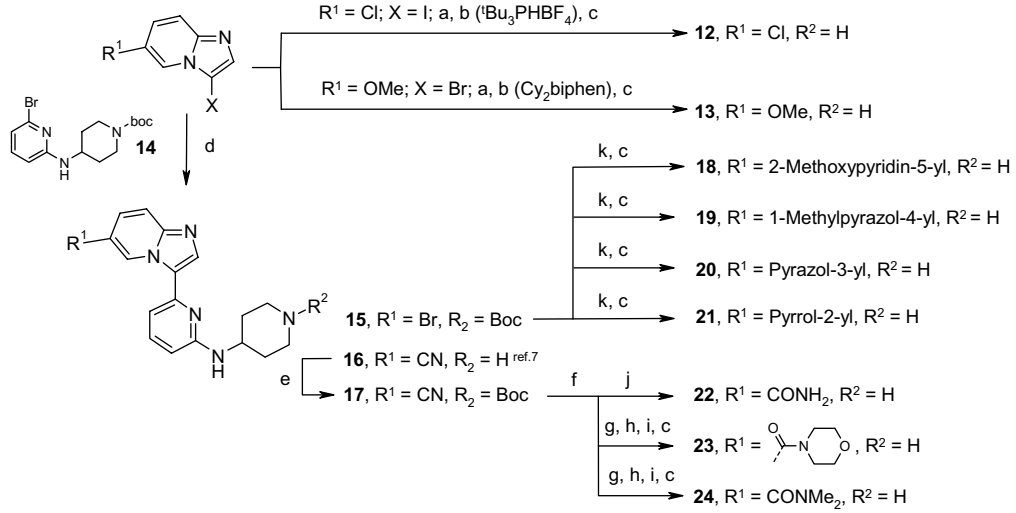
Table 1. IRAK-4 enzyme inhibition for benzimidazolo-pyridines **3–11**

Compound	R^1	R^2	IRAK-4 inhibition, IC_{50} (nM)
3	H	H	70
4	H	OMe	15
5	OMe	H	208
6	OMe	OMe	181
7	H	Cl	3
8	Cl	H	112
9	Cl	Cl	166
10	H	Me	10
11	Me	H	1085

couplings (method c).[5] Bifunctional bis-amines were suitably protected with *tert*-butoxycarbonyl groups. The products were purified by mass directed preparative HPLC[6] following deprotection step (d) where required.

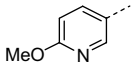
The series of aniline and heteroaryl analogues (**26**–**29**) showed some interesting SAR (e.g., 2-PhCN > Ph ~ 2-*N*-methylpyrazol-3-yl > 4-PhCN), albeit with modest potencies (Table 3). Some examples of secondary linked alkylamines indicated a preference for a free –NH–/NH_2 spaced approximately 2–3 carbon atoms from the linking amino group (e.g., **32** > **31** and **34** > **33**). The IRAK-4 potency varied markedly with quite subtle changes to the positioning of the distal amino group. For example, the bicyclic analogue **34** was an order of magnitude less potent than 4-aminopiperidine, **2**, whilst azetidine **37** and 3-(*S*)-piperidyl **39** had comparable potency. The 3-linked pyrrolidine ***rac*–38** and methylene spaced 2-linked pyrrolidine ***rac*-36** were ~10-fold less potent than **2** and the methylene spaced 4-linked piperidine **35** is 100-fold less potent. Tertiary N-linked alkylamines (**40**–**43**) demonstrated that a 2-aminopyridine free NH was not an essential prerequisite to binding, consistent with our earlier findings.[1] However, these compounds do not represent especially potent IRAK-4 inhibitors. Once again, the precise positioning and constraints of the distal free amino NH/NH_2 appeared to be important for good IRAK potency (***rac*-42** > **41**).

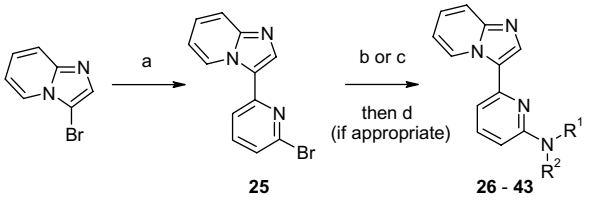
Having assessed the SAR of substituents at different ends of the molecule the obvious next step was to combine preferred substituents in the same structure (Table



Scheme 2. Synthesis of 6-substituted imidazo[1,2-*a*]pyridin-3-yl-pyridin-2-ylamines, **12**–**24**. Reagents and conditions: (a) *i*-PrMgCl, THF, −78 °C; *n*-Bu_3SnCl, −78 °C to rt; 2,6-dibromopyridine, $Pd(PPh_3)_4$, 140 °C, microwave (11–21%); (b) 4-amino-1-Boc piperidine, NaO^tBu, $Pd(OAc)_2$; *t*-Bu_3PHBF_4 DME, 140 °C, microwave (47%) or 2-(dicyclohexylphosphino)biphenyl (Cy_2biphen), toluene, 140 °C, microwave (44%); (c) HCl, Et_2O, DCM, MeOH (40–63%); (d) *i*-PrMgCl, THF, −78 °C; *n*-Bu_3SnCl, −78 °C to rt; **14**, $Pd(PPh_3)_4$, reflux or 140 °C, microwave;[7] (e) Boc_2O, Et_3N, DCM (61%); (f) KOH, THF, H_2O, 140 °C, microwave; (g) 20% (aq) H_2SO_4, 140 °C, microwave (57%); (h) Boc_2O, NaOH, dioxane, H_2O (96%); (i) morpholine or dimethylamine (2.0 M in THF), EDCI, HOBt, DCM (38–43%); (j) TFA, DCM (57%); (k) $ArB(OH)_2$ or $ArB(OCMe_2)_2$, Na_2CO_3, *n*-Bu_4NBr, $Pd(PPh_3)_4$, DME, H_2O, 140 °C, microwave (47–50%).

Table 2. IRAK-4 enzyme inhibition for 6-substituted imidazo-[1,2-*a*]pyridin-3-yl-pyridin-2-ylamines, **12–24**

Compound	R^1	IRAK-4 inhibition, IC_{50} (nM)
12	Cl	1
13	OMe	6
16	CN	4
18		8
19		7
20		2
21		63
22		5
23		263
24		49



Scheme 3. Parallel synthesis of 2-amino substituted imidazopyridino-pyridines **26–43**. Reagents and conditions: (a) *i*-PrMgCl, THF, −78 °C; *n*-Bu_3SnCl, −78 °C to rt; 2,6-dibromopyridine, $Pd(PPh_3)_4$; 66 °C (71%); (b) R^1R^2NH, Et_3N, *n*-BuOH, 150 °C, microwave; or (c) R^1R^2NH, $Pd(OAc)_2$, Cy_2biphen, NaO^{t}Bu, toluene, 150 °C, microwave; (d) HCl, Et_2O, DCM (77–97%).

4). The compounds were prepared by constructing the substituted imidazopyridine from condensing functionalised 2-aminopyridines with chloroacetaldehyde[8] and halogenating regioselectively in the 3-position with *N*-iodo or *N*-bromosuccinmide.[9] Once again, Stille cross-coupling and Buchwald conditions were used to construct the final compounds (Scheme 4).

In the examples given it was apparent that the SAR was not additive. Having a substituent in the imidazopyridine 6-position generally increased potency by 4- to 500-fold (**44–47**). This perhaps suggested the relative dominance of the 6-substituent in terms of binding over the pendant 2-amino heterocycles. However, compound **48** is an example where this is not the case. In the absence of a 6-substituent (R^1 = H, **39**), potency is a respectable 19 nM. Introduction of the nitrile group in the 6-position dropped potency by ~5-fold. This high-

Table 3. IRAK-4 enzyme inhibition for 2-amino substituted imidazo-pyridino-pyridines **26–43**

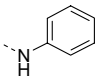
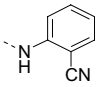
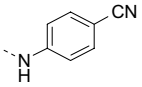
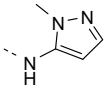
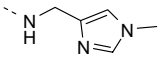
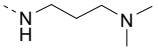
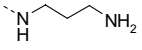
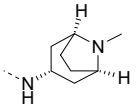
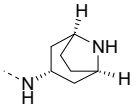
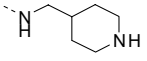
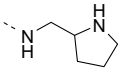
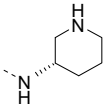
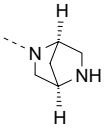
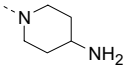
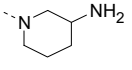
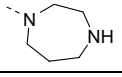
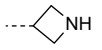
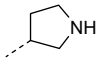
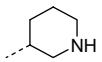
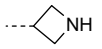
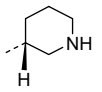
Compound	NR^1R^2	IRAK-4 inhibition, IC_{50} (nM)
26		1156
27		433
28		(12% inhibition at 10 μM)
29		1134
30		497
31		8100
32		1300
33		914
34		225
35		3030
***rac*-36**		313
37		39
***rac*-38**		545
***abs*-39**		19
***abs*-40**		126
41		1880
***rac*-42**		535
43		487

Table 4. IRAK-4 inhibition of hybridized substituted imidazo[1,2-*a*]-pyridino-pyridines **44–48**

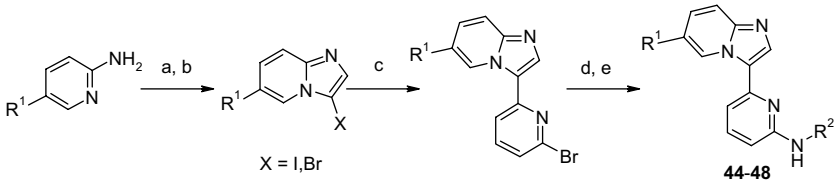
Compound	R^1	R^2	IRAK-4, IC_{50} (nM)
44	Cl		6
***rac*-45**	Cl		1
***rac*-46**	Cl		9
47	OMe		7
48	CN		95

lights subtleties in SAR, where optimising two remote parts of a molecule independently sometimes results in non-additive effects.

Compounds that exhibited significant IRAK-4 potency were submitted into a screening cascade to build up a fuller profile and to prioritise compounds for in vivo studies (Table 5). This included an IRAK-1 enzyme assay.[10] IRAK-1 is a related kinase homologue that is implicated in the same signalling pathway and hence a dual inhibitor of both IRAK-4 and IRAK-1 could offer synergistic benefits.[11] A variety of functional cell assays were also used to assess potency, such as LPS-induced TNFα cytokine release assay.[12] Potency in the IRAK enzyme assays tracked reasonably well with cytokine release in the cell assays. Good cellular IC_{50}s of 10s–100s nanomolar were often observed. The physicochemical profile of this series of compounds was generally good, with few solubility issues,[13] and the in vitro DMPK looked promising with low to moderate microsomal clearance in both human and rat. Cytochrome P_{450} liabilities were not a major issue, with many compounds exhibiting only very weak CYP inhibition.[14]

We were encouraged by the fact that this series of compounds exhibited high enzyme potencies (frequently <10 nM at 10 μM ATP), whilst still retaining low molecular weights (300–350). This represents high Ligand Efficiency (LE)[15] and suggests further optimisation could be achieved, designing in selectivity elements whilst retaining IRAK potency and desirable physicochemical properties. For example, compound **16** has a LE of 0.52 (Table 5). As a point of reference the average kinase inhibitor has a LE of 0.3.[16] The calculated Log *P* and PSA are all respectably low ($a\text{Log}\,P < 3$, PSA < 85Å^2), giving plenty of scope for further elaboration.[17]

We have described how an active hit identified through routine cross screening was developed into a new class of IRAK-4 inhibitor. The SAR of the new lead series was explored with guidance from the IRAK-4 homology model developed in-house. Some highly potent (low nanomolar) IRAK-4 inhibitors with good cellular TNFα inhibition were prepared and were shown to have good drug-like properties and encouraging in vitro DMPK profiles. Initial kinase panel profiling and cell screening has indicated that some degree of off-target activity is still evident with this series, despite departing from the aminopyrimidine motif. However, the scope for fine tuning and optimising this potent and efficient class of IRAK inhibitor could lead to the generation of new therapeutic agents or useful tool compounds based on the blockade of the IL-1 driven immune response.



Scheme 4. Synthesis of hybridised substituted imidazo[1,2-*a*]pyridino-pyridines **44–48**. Reagents and conditions: (a) chloroacetaldehyde, $NaHCO_3$, H_2O (45–55%); (b) NIS or NBS, MeCN (18–64%); (c) i-PrMgCl, THF, −78 °C; *n*-Bu_3SnCl, 66 °C; 2,6-dibromopyridine, $Pd(PPh_3)_4$, 150 °C, microwave; (28–44%); (d) Boc-R_2–NH_2, $Pd(OAc)_2$, dicyclohexyl-1-biphenylphosphine, NaO^{t}Bu, microwave 140 °C toluene; (e) HCl, DCM, Et_2O, MeOH.

Table 5. Selected in vitro/cellular potency, in vitro DMPK data and calculated properties

Compound	MWt	2D PSA[a]	$a\text{Log}\,P$[a] (Å^2)	IRAK-4 IC_{50} (nM)	Ligand efficiency (LE)[b]	IRAK-1 IC_{50} (nM)	TNFα inhibition (nM)	Mic. Clint, (hu) at 0.5 μM[c]	Mic. Clint, (rat) at 0.5 μM[c]	CYP 2D6 inhibition (μM)	CYP 3A4 inhibition (μM)
13	323	68.1	1.7	6	0.52	293	220	19.5	5.3	41% at 50 μM	31
16	318	82.6	1.6	4	0.54	96	200	8.3	39.9	42	22
44	300	55.5	2.3	6	0.48	195	54	13	0.01	21% at 10 μM	11
***rac*-45**	314	60.1	2.3	1	0.48	23	16	19.2	49.3	15	12
***rac*-46**	328	60.1	2.9	9	0.47	175	65	29.5	78.1	1.8	8.6

[a] *a*log *P* and 2D PSA calculated with Scitegic Pipeline Pilot software. http://www.scitegic.com.
[b] Ligand efficiencies approximated from IC_{50} values: LE (kcal mol^{-1}) = {[−RT ln IC_{50} (mol L^{-1})]/# of non-hydrogen atoms}.[15]
[c] Units for micosomal intrinsic clearance, μL/min/mg of protein.

Acknowledgements

The authors thank the analytical support group. In particular Jags Matharoo (mass directed preparative HPLC), Paloma Fernández Díaz (LC–MS) and Phil Gilbert (NMR). We also thank the DMPK group for performing the microsome stability and CYP inhibition assays (Andy Sykes and Helen Brown) and the protein expression group (David Rainey and Alistair Durrant) for supplying IRAK-1 and IRAK-4 for the enzyme assays and crystallographic trials.

References and notes

1. Buckley, G. M. et al. *Bioorg. Med. Chem. Lett.* **2008**, doi:101016/j.bmcl.2008.04.039.
2. An example of a similar chemotype to the benzimidazolo pyridines has been reported by Pfizer as a PDGFR inhibitor (CP-673,451) but no binding hypothesis is offered. *Cancer Res.* **2005**, *65*, 957.
3. (a) Stille, J. K. *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 508; (b) Farina, V.; Krishnamurthy, V.; Scott, W. J. *Org. React.* **1998**, *50*, 1; (c) Mitchell, T. N. *Synthesis* **1992**, 803.
4. Biotage (formerly Personal Chemistry) Emrys Optimizer™. Reaction times varied from 5 min to >1 h. Individual reactions were periodically monitored by LC–MS until complete conversion or emergence of undesired by-products.
5. Wolfe, J. P.; Buchwald, S. L. *Org. Synth.* **2004**, *10*, 423; Wolfe, J. P.; Buchwald, S. L. *Org. Synth.* **2002**, *78*, 23.
6. Waters Autoprep. Mass (ESI positive ion mode) and u.v. directed fraction collection. Phenomenex Luna® stationary phase (5μ C18(2); 250 × 21.2 mm) against acetonitrile gradient with either formic acid (0.8 vol %) or ammonium acetate (10 mM) mobile phase.
7. Under the high temperature (140 °C) Suzuki coupling conditions compound **16** (R_1 = CN) was formed directly with loss of the Boc protecting group whilst compound **15** (R_1 = Br) retained the Boc group. This may be due to relative substrate stabilities or subtle experimental variations between the two reactions. In any case, reinstallation and removal of the Boc groups was synthetically trivial.
8. Löber, S.; Hübner, H.; Gmeiner, P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 97.
9. Enguehard, C.; Renou, J.-L.; Collot, V.; Hervet, M.; Rault, S.; Gueiffier, A. *J. Org. Chem.* **2000**, *65*, 6572, Compound **47** derived via NBS bromination (X = Br) whilst compounds **44–46** and **48** derived via analogous NIS iodination (X = I).
10. The IRAK-1 in vitro enzyme assay determined the effect of test compounds on the phosphorylation of a biotinylated 32 amino acid peptide based on the activation loop of IRAK1, using FlashPlates to detect incorporation of ^{33}P. It was performed in 96-well streptavidin coated FlashPlates in a volume of 100 µl, comprising of reaction buffer (50 mM Hepes, 10 mM $MgCl_2$, 5 mM EGTA, 1 mM DTT, pH 7.2), 1 µM ATP, 0.5µCi/well ^{33}P-ATP, 2 µM peptide substrate (Biotin-DFGLARFSRFAGSSPSQSSMVARTQ-TVRG TLA, Peptide Protein Research Ltd), 8 nM full length GST-His-IRAK1 (in-house) and test compound in 2% DMSO. The kinase reaction was incubated for 90 min at room temperature, then terminated by addition of 100 µl 100 mM EDTA. After a further 30 min incubation to maximise peptide capture, the plate was washed three times with 0.1% Tween 20 in PBS. Incorporation of ^{33}P into the peptide substrate was measured using a TopCount plate reader.
11. Lye, E.; Mirtsos, C.; Suzuki, N.; Suzuki, S.; Yeh, W. C. *J. Biol. Chem.* **2004**, *279*, 40653.
12. Peripheral blood mononuclearcytes (PBMC) were isolated form human blood by density gradient centrifugation. Cells were treated with IL-1β in the presence of test compounds for 24 h. Supernatants were collected and the levels of soluble cytokines analysed via multiplexed particle based flow cytometric assay (Luminex).
13. Solubility measured by AKAS determination from DMSO stock solutions. Of 44 compounds from this article that were tested, 40 had >999 µM solubility at pH 7.4.
14. Hollenberg, P. F. *Drug Metab. Rev.* **2002**, *34*, 17.
15. Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discovery Today* **2004**, *9*, 430.
16. Paolini, G. V.; Shapland, R. H.; van Hoorn, W. P.; Mason, J. S.; Hopkins, A. L. *Nat. Biotechnol.* **2006**, *24*, 805.
17. (a) Leeson, P. D.; Springthorpe, B. *Nat. Rev. Drug Disc.* **2007**, *6*, 881; (b) Lipinski, C.; Lombardo, F.; Dominy, B.; Feeney, P. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.